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Lessons learned from the contamination of heparin

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Abstract

Heparin is unique as one of the oldest drugs currently still in widespread clinical use as an anticoagulant, a natural product, one of the first biopolymeric drugs, and one of the few carbohydrate drugs. Recently, certain batches of heparin have been associated with anaphylactoid-type reactions, some leading to hypotension and death. These reactions were traced to contamination with a semi-synthetic oversulfated chondroitin sulfate (OSCS). This *Highlight* reviews the heparin contamination crisis, its resolution, and the lessons learned. Pharmaceutical scientists now must consider dozens of natural and synthetic heparinoids as potential heparin contaminants. Effective assays, which can detect both known and unknown contaminants, are required to monitor the quality of heparin. Safer and better-regulated processes are needed for heparin production.

Introduction

A highly sulfated glycosaminoglycan, heparin is widely used as an *intravenous* anticoagulant and has the highest negative charge density of any known biological molecule.^{1–3} Heparin is unique as one of the oldest drugs currently still in widespread clinical use, one of the first biopolymeric drugs, and one of the few carbohydrate drugs.³ Recently, a new, rapid onset, acute side effect associated with heparin was reported, which was believed to be caused by an anaphylactoid response, leading to hypotension and resulting in nearly 100 deaths.^{4,5} This crisis in heparin-based therapy has received enormous attention. This *Highlight* reviews the history and structure of heparin, together with its biological and pharmacological (anticoagulant and antithrombotic) activities. Current methods for heparin production from animal tissues are described. The contamination crisis and reliable methods to detect contaminants in heparin and low molecular weight heparin are reviewed. Finally, new methods under development are described that should ensure the future safety of heparin products.

Heparin structure and activities

The heparin story^{2,6,7} began during World War I in 1916 at Johns Hopkins University in Baltimore. Jay McLean, a second-year medical student working under the direction of William Howell, isolated fractions from mammalian tissues that inhibited blood coagulation.

After some initial skepticism, Howell, recognizing the importance of the discovery of his student, suggested using this isolate to treat coagulation disorders. An understanding of heparin's structure developed slowly. Howell determined that heparin had no phosphate and was a carbohydrate. Sune Bergstrom, winner of the Nobel Prize for research on prostaglandins, correctly identified glucosamine (GlcN) as a sugar component in heparin while working as a student working with Eric Jorpes in Sweden. Jorpes established that heparin contained a high proportion of sulfo groups, making it one of the strongest acids in nature, and determined that the GlcN residue in heparin was primarily *N*-sulfonated. Melville Wolfrom initially identified the uronic acid residue as D-glucuronic acid (GlcA), Tony Cinfonelli and Al Dorfman reported L-iduronic acid (IdoA) in heparin, and in 1968 Arthur Perlin correctly identified IdoA as the major uronic acid residue in heparin using NMR spectroscopy. By the 1920s, several groups were manufacturing heparin. In Toronto, Charles Best, a colleague of Banting (both winners of the Nobel Prize for discovering insulin), began a research program aimed at the commercial production of heparin from bovine lung and later porcine intestine. Eric Jorpes transferred this technology from Toronto to Stockholm and by 1935, Jorpes in Stockholm, along with Arthur Charles and David Scott of Connaught Laboratories at the University of Toronto, had prepared sufficient heparin for human trials. In the 1930s, Gordon Murry in Toronto and Clarence Crafoord in Stockholm successfully began using heparin in surgery patients, a medical practice that continues to this day.

Like all other natural polysaccharides, heparin is a polydisperse mixture containing a large number of chains having different molecular weights.^{8,9} The polydispersity (the ratio of weight averaged to number averaged molecular weight) of pharmaceutical heparin is 1.1–1.6.⁸ The chains making up polydisperse pharmaceutical grade heparin range from 5,000 to over 40,000 Da³ and contain a significant level of sequence heterogeneity. Heparin is composed of a major (75–95%) trisulfated disaccharide repeating unit (Fig. 1A), a 2-*O*-sulfo- α -L-iduronic acid 1 $\rightarrow$ 4 linked to 6-*O*-sulfo-*N*-sulfo- α -D-glucosamine ($\rightarrow$ 4)IdoA2S(1 $\rightarrow$ 4)GlcNS6S[1 $\rightarrow$), as well as a number of additional minor disaccharides structures corresponding to its variable sequences (Fig. 1B).^{10–12} There are some fully sulfated heparin chains that are simply composed of uniform repeating sequences of trisulfated disaccharide. Most heparin chains, however, have an intermediate level of sulfation (2.5 sulfo groups/disaccharide) and are composed of long segments of fully sulfated sequences with intervening undersulfated domains. Some chains, primarily composed undersulfated sequences, are classified as heparan sulfate, a closely related glycosaminoglycan. A unique saccharide combination comprises the antithrombin III (ATIII) pentasaccharide binding site, GlcNAc/NS6S $\rightarrow$ GlcA $\rightarrow$ GlcNS3S,6S $\rightarrow$ IdoA2S $\rightarrow$ GlcNS6S, important for heparin's anticoagulant activity (Fig. 2).^{13,14}

Heparin and the structurally related heparan sulfate participate in numerous important biological processes such as blood anticoagulation, pathogen infection, cell differentiation, growth and migration and inflammation.^{15–20} Heparin carries out its biological functions primarily by its interaction with proteins in which heparin's sulfo groups electrostatically interact or hydrogen bond with basic amino acids of the target protein.^{1,21,22} Heparin can bind heparin-binding proteins specifically and with high affinity, as dictated by the number and relative position of the sulfo groups in its interacting oligosaccharide sequence.²³ ATIII binds to a specific ATIII pentasaccharide sequence in heparin.^{13,14} When heparin binds to the serine protease inhibitor ATIII, it undergoes a conformational change resulting in the inhibition of thrombin and other coagulation cascade proteases.^{22,24} Only a third of the chains comprising pharmaceutical-grade heparin contain an ATIII binding site, and these are called “high affinity heparin”.^{3,24,25} In contrast, heparin interacts with low specificity to thrombin based on its high negative charge density. Thus, if a heparin chain containing an ATIII binding site is sufficiently long to accommodate thrombin, it can form a tertiary

complex, inhibiting thrombin's conversion of soluble fibrinogen to an insoluble fibrin clot. Low molecular weight heparins (LMWHs) are prepared through the controlled chemical and enzymatic depolymerization of heparin.²⁶ LMWH chains are often too small to accommodate thrombin in a ternary complex, and thus inhibit the coagulation cascade primarily through coagulation factor Xa. The clinical value of LMWHs comes primarily from their enhanced *subcutaneous* bioavailability.²⁴

The production of heparin from natural sources

Heparin and other glycosaminoglycans are generally isolated by extraction from animal tissues, but some simple unsulfated glycosaminoglycans can be obtained from the capsules of bacteria.^{27–30} Heparins from tissues of various species also differ in structure and activity (Table 1).¹¹ For example, porcine intestinal heparin has an ATIII binding site primarily containing an *N*-acetyl (NAc) group,^{11,31} while bovine lung heparin primarily contains an *N*-sulfo (NS) group (Fig. 2, residue 1), resulting in slight differences in their affinities for ATIII.^{11,24}

Pharmaceutical heparins are most commonly isolated in tons quantities from porcine intestines.³² The disaccharide composition of individual porcine intestinal heparins can also differ (Table 1).¹⁰ Some porcine intestinal heparin is prepared from porcine intestinal mucosa, scraped from the intestine, while other preparations use the whole intestine (“hashed pork guts”). These two raw materials contain differing amounts of structurally related heparan sulfate that can carry over into the final pharmaceutical product. There are also different subspecies of pigs, and the mast cell content of intestinal tissue can vary based on the diet and environment in which the animals are raised. These variables potentially contribute to the already complex structure of pharmaceutical-grade heparin.

Methods of commercial production of pharmaceutical grade heparin are tightly guarded industrial secrets, and few publications or patents describe commonly used pharmaceutical processes. The process of preparing pharmaceutical grade heparin has been altered somewhat over time as the primary tissue source has changed from dog liver to beef lung and finally to porcine intestine.³² Large-scale commercial processes are typically used to prepare multi-kilogram batches of pharmaceutical grade heparin to accommodate the 100 tons used each year worldwide. The method used today for the commercial preparation of porcine intestinal heparin has changed little from that used early this century, and involves five basic steps: 1) preparation of tissue; 2) extraction of heparin from tissue; 3) recovery of raw heparin; 4) purification of heparin; and 5) recovery of purified heparin.

The preparation of the tissue begins with collection at the slaughterhouse and its preparation for processing (Fig. 3A). In the past, whole intestine (“hashed pork guts”) or mucosa were transferred from the slaughterhouse to a heparin manufacturing facility for further processing. Now, however, raw heparin extraction typically takes place at the pig slaughtering facility itself (*not* under current good manufacturing practices (cGMP) conditions), to minimize the environmental impact of high-ash, high-biochemical-oxygen-demand hydrolyzed protein.³³ Additional high potency heparin can be recovered by saving the waste brine solution of the pig casings operation.³⁴

Older processes used a quaternary ammonium salt³⁵ to form a complex with the heparin-like GAGs present in the filtrate. This process has largely been replaced to avoid the negative impact of the bactericidal activity of quaternary ammonium compounds on wastewater treatment plants.

The purification of the resulting raw heparin is performed under cGMP conditions and is designed to deal with potential impurities originating from the starting material or

introduced during raw heparin extraction (Fig. 3B). Such impurities may be in the form of other GAGs, extraneous cationic counterions, heavy metals, residual protein or nucleotides, solvent, salts other than heparin, bacterial endotoxins, bioburden, and viruses. Specific commercial methods of purifying heparin are tightly guarded industrial secrets set forth in a Drug Master File submitted to the FDA and foreign regulatory authorities. The yield of USP heparin from American pigs is typically 30,000–50,000 U, corresponding to ~300 mg per animal (Chinese pig intestine contains a slightly higher level of heparin). Small amounts (1–7%) of dermatan sulfate is present in some pharmaceutical grade heparins, but can be virtually eliminated using good manufacturing processes.^{10,36}

The heparin contamination crisis

In early 2008 there was a marked increase in serious adverse events associated with heparin therapy, with thousands of patients affected (Fig. 4). These events included development of such symptoms as rash, fainting, racing heart, and other more severe symptoms. This has resulted in the death of nearly 100 Americans and a national healthcare crisis. Nations in the European Union and Asia also observed a similar phenomenon, making this an international issue. The rapid onset of these symptoms suggests an anaphylactic response but the exact etiology is currently unknown. The contaminated lots of heparin were voluntarily withdrawn from the US market in March 2008, and as a result by April 2008 the number of adverse reactions returned to the background level reported to the FDA in April 2007 (preceding the heparin contamination). It was at this point that an international consortium of laboratories was enlisted to work with the FDA and the pharmaceutical industry to identify the contaminant and to help correlate its presence to the reported adverse events. Several analytical tests began to detect differences in suspect *versus* control lots.³⁷ Screening of heparin lots by a combination of optical rotation, capillary electrophoresis (CE), and 1D ¹H-NMR indicated a defined pattern that could be used to distinguish suspect from control lots. In the case of capillary electrophoresis, suspect lots contained an additional, leading edge peak in addition to the broad peak associated with heparin. Proton NMR analysis indicated distinctive differences between suspect and control lots, most prominently in the acetyl region of the spectrum (2–2.2 ppm) (Fig. 5). Given the nature of these analyses, and the differences observed upon comparison of suspect *versus* control lots, the source of the major contaminant was surmised to be a highly sulfated “heparin-like” contaminant.³⁷ The investigation of the identity of the contaminant converged on an oversulfated chondroitin sulfate (OSCS) first synthesized in 1998 by Linhardt and Toida and coworkers.³⁸ OSCS is synthesized by the chemical sulfonation of chondroitin sulfate, an inexpensive (<10% of the cost of heparin) nutraceutical used for the self-treatment of osteoarthritis.³⁹ This semi-synthetic polysaccharide had a molecular weight of 18 kDa, comparable to heparin, and a slightly higher charge density (–5 (sulfo + carboxyl groups)/disaccharide for OSCS compared to –3.7/disaccharide for heparin). The ¹H-NMR and optical rotation of OSCS, synthesized 10 years earlier, was similar to the contaminant isolated from heparin, as was its anti-FIIa activity.³⁹ Thus, the structure of the contaminant was confirmed and reported in *Nature Biotechnology*.⁴⁰ LMWH was also found to be contaminated. The stability of OSCS in heparin processed into LMWH depended on the nature of the depolymerization chemistry (Fig. 6).⁴¹

Biological and pharmacological effects of contaminated heparin

The reason for the rapid-onset, acute side effect associated with heparin was determined to involve an anaphylactoid response⁴ (Fig. 7). This resulted in a spike in adverse events ascribed to certain contaminant lots of heparin. Analysis of these lots revealed the presence of the semi-synthetic OSCS. Both the isolated contaminant and independently synthesized OSCS activated the kinin–kallikrein pathway in human plasma, leading to bradykinin

formation. OSCS was also shown to induce complement protein C3a and C5a generation in the complement cascade. The activation of the kinin–kallikrein and complement pathways was linked through fluid-phase activation of FXII in the coagulation cascade. The kallikrein–kinin pathway starts with human plasma FXII. When exposed to a negatively charged surface and damaged endothelial cells, FXII (the inactive serine protease precursor) is activated to FXIIa (the active serine protease), which is able to cleave prekallikrein into kallikrein. This results in the production of the potent vasoactive mediator bradykinin and the complement-derived anaphylatoxins. FXII, prekallikrein and high molecular weight kininogen form a ternary complex capable of forming bradykinin. Thus, the kinin–kallikrein, complement and coagulation pathways suggested an explanation for the anaphylactoid response observed in patients intravenously administered OSCS-contaminated heparin. Screening of plasma samples from various species indicated that pigs and humans are sensitive to the effects of OSCS in a similar manner. A recent study by our laboratory found that the anticoagulant activity of this OSCS chondroitin sulfate involves heparin cofactor II (HCII)-mediated inhibition of thrombin.^{38,42} Heparin and OSCS binding to coagulation, kinin–kallikrein and complement proteins were determined using surface plasmon resonance. While OSCS binds tightly to ATIII, unlike heparin, OSCS does not induce ATIII to undergo the conformational change required for its inactivation of thrombin and FXa. In contrast to heparin, OSCS tightly binds FXIIa, suggesting a mechanism for the further enhancement of vaso-active bradykinin production resulting in the hypotensive side effects observed in patients and pigs administered contaminated heparins.^{38,42}

There are current concerns about the clearance of OSCS from the body, particularly in kidney dialysis patients that received multiple doses of contaminated heparin and have low renal clearance function. Long-term exposure to OSCS might lead to chronic toxicity in the large number of patients who received contaminated heparin.

Identification and testing of contaminants and impurities

OSCS-contaminated heparins were first distinguished from control heparins by optical rotation, CE, and ¹H-NMR.³⁷ Furthermore, the structure of OSCS was confirmed using NMR (¹H, ¹³C, COSY, HSQC and HMQC) coupled with LC-MS.⁴⁰ The ¹H-NMR spectrum of contaminated heparin sample shows more than the 16 proton signals expected for pure heparin (Fig. 5). The signals associated with OSCS were all present at lower fields, confirming the fully sulfated structure of the contaminant. After desulfonation, chondroitinase treatment afforded chondroitin oligosaccharides as confirmed by LC-MS.

New quantitative methods of heparin analysis are currently under development. NMR still represents the most reliable method to detect OSCS and other contaminants and impurities. Both OSCS and DS can be quantified using ¹H-NMR.⁴³ The acetyl group signals of heparin, DS and OSCS resonate at 2.05, 2.08, and 2.15 ppm, respectively, and are easily quantified based on their integration. As little as 0.1% DS impurity and OSCS contaminant is detectable by ¹H-NMR. In addition to OSCS, dozens of natural or artificial heparinoids having charge properties, molecular weight and anticoagulant activities similar to heparin have been described over the past 30 years (Table 2).^{44,45} Thus, the risk of contamination or adulteration of heparin still exists. A ¹H-NMR screen of these common heparinoids mixed with pharmaceutical heparin has recently been undertaken in our laboratory. All of these potential heparinoid contaminants could be readily identified based on their ¹H-NMR spectra. NMR has become a critical analytical method for the control of heparin prepared from animal tissues.

Other assays also play a role in keeping the heparin supply safe. OSCS blocks the activity of Taq polymerase used for real-time PCR.⁴⁶ Based on this finding, a simple, rapid and

sensitive method was developed for high-throughput screening to detect and quantify OSCS and other potential oversulfated contaminants in commercial lots of heparin. This method requires 0.1 units of heparin and has a limit of detection for OSCS of <1 ng. OSCS is stable to many conditions used to depolymerize heparin, including treatment with heparin lyase, nitrous acid and sodium periodate (Fig. 6).⁴¹ Nitrous acid treatment is a simple and specific method to recover intact OSCS from heparin; the *N*-acetylgalatosamine residue in OSCS is insensitive to nitrous acid while the *N*-sulfoglucosamine residue in heparin is deaminatively cleaved. The remaining OSCS can then be quantified using a variety of assay methods. Most natural heparinoids (with the exception of HS) and chemically modified heparinoids (with the exception of chitosan sulfate) can be detected using nitrous acid. Unfortunately, once nitrous acid becomes widely used to detect contaminants/adulterants, undetectable adulterants such as *N*-sulfo-OSCS or *N*-sulfo-per-*O*-sulfo-hyaluronan may be introduced to avoid detection. The use of more specific heparin lyases might address this limitation.

New approaches for preparing heparin from non-animal sources

Chemical synthesis is the first alternative that comes to mind when looking for new ways to produce safe heparin. Sanofi introduced a synthetic heparin pentasaccharide drug called Arixtra® (fondaparinux sodium) in 2002 (Fig. 8).⁴⁷ This drug was based on the simplification of the elegant synthesis of the heparin ATIII pentasaccharide binding site first reported by Choay and coworkers in 1980s.⁴⁸ Arixtra differs from heparin in that it is a specific anti-FXa agent, and that it does not have many of the important pharmacological properties of the polycomponent drug heparin.⁴⁹ It is interesting to note that when Sanofi merged with Aventis in 2004, Sanofi-Aventis sold the rights to the defined Arixtra pentasaccharide to GSK and retained the rights to the Aventis polycomponent LMW heparin-derived product Lovenox® (enoxaparin sodium). This decision was based primarily on the economics of production of drugs, their perceived pharmacological advantages, and the projected market share of both drugs. This decision was clearly the correct one, with the LMWH Lovenox having captured ~70% of the \$3 billion heparin product market share in the US, compared to ~3% for the synthetic Arixtra. Unlike heparin, Arixtra has a long half-life, and the excess anticoagulant activity cannot be reversed by protamine. Further, Idraparinux, a new pegylated Arixtra analog, exhibited a high-risk bleeding effect due to its excessively long half-life in a clinical trial.⁵⁰ Thus, a fully synthetic heparin pentasaccharide is not currently viewed as a viable alternative to heparin.

Extensive studies by Lindahl and co-workers have shown how heparin and heparan sulfate are biosynthesized (Fig. 9).⁵¹ A core protein is synthesized in the endoplasmic reticulum with a tetra-saccharide linker attached to its serine residues in regions rich in Ser-Gly repeats.⁵¹ As it transits the Golgi, a repeating 1 → 4-glycosidically-linked copolymer of D-glucuronic acid and *N*-acetyl-D-glucosamine is extended from this linkage region, through the stepwise addition of uridine diphosphate-activated sugars catalyzed by EXT enzyme.⁵³ During its formation, the linear homocopolymer is sequentially modified through the action of *N*-deacetylase/*N*-sulfotransferase (NDST), C-5 epimerase (C5Epi), and 2-, 6-, and 3-*O*-sulfotransferases (OSTs). Complete or nearly complete modification of this nascent GAG chain results in a GAG rich in *N*- and *O*-sulfo-L-iduronic acid, 'heparin'. Partial modification of the same chain results in a GAG rich in *O*-sulfo-*N*-acetyl-D-glucosamine and D-glucuronic acid, 'heparan sulfate'.

Over the past decade a European consortium has worked on a chemoenzymatic alternative to heparin called "neoheparin".⁵⁴ This approach begins with *E. coli* K5 as a source of the polysaccharide backbone, which is chemically de-*N*-acetylated and *N*-sulfonated. This *N*-sulfoheparosan is treated with C5Epi, chemically per-*O*-sulfonated and then selectively *O*-desulfonated. The resulting neoheparin product contains unnatural sequences not found in

mammalian heparin, including 3-*O*-sulfoglucuronic acid. In light of the current heparin contamination crisis involving OSCS, also with unnatural 3-*O*-sulfoglucuronic acid residues, the introduction of neoheparin poses some concerns.

Recently, our laboratory chemoenzymatically reconstructed a heparin in milligram quantities by following the heparin biosynthetic pathway.^{55–57} All of the enzymes required for the biosynthesis of heparin and heparan sulfate have been cloned and expressed. Using two simple chemical steps, followed by four enzymatic steps, a bioengineered heparin was chemoenzymatically synthesized from *E. coli* K5 *N*-acetyl heparosan (Fig. 10).⁵⁶ Uniform ¹³C and ¹⁵N isotope labeling could also be introduced into this heparin to further assist structural analysis. The anticoagulant activity of chemoenzymatically synthesized precursor to heparin was 20 ± 6 U/mg, while the final bioengineered heparin had an anticoagulant activity of 180 ± 15 U/mg. This activity was comparable to the 170 U/mg displayed by a standard pharmaceutical USP heparin. The ATIII binding, anti-Xa and anti-IIa activities of this heparin were also determined, and were comparable to those of USP heparin.⁵⁸ By this method, it might one day be possible to prepare the kilogram quantities of bioengineered heparin required to support human clinical trials and ultimately ton-scale quantities required to replace animal-sourced heparin.

Conclusion

Heparin has been one of the most effective and widely used drugs of the past century. Predating the establishment of the US Food and Drug Administration, it is one of the oldest drugs currently still in widespread clinical use, and it is unique because it was among the first biopolymeric drugs, and is one of only a few carbohydrate drugs. However, the recent contaminant crisis has brought heparin back to our attention. This polydisperse, poly-component, polypharmacologic agent is difficult to monitor and to analyze, but the more we understand it the lower will be the risk of future crises in its production and use. Therefore, alternative methods of controlling and regulating the production of heparin are urgently needed, and such procedures might represent the best long-term solution.

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Biographies

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Fig. 1.

The structures of the major (**A**) and minor (**B**) repeating disaccharides comprising heparin where $\text{X} = \text{SO}_3^-$ or H , and $\text{Y} = \text{SO}_3^-$ or COCH_3 .

Fig. 2.
Heparin's antithrombin III binding site and its structural variants.

Fig. 3.

Flow chart of the preparation of heparin from animal tissues. Chart **A** is performed under little or no regulatory control in rural communities while chart **B** is performed under cGMP in FDA-inspected facilities.

Fig. 4.
Timeline of the heparin crisis.

Fig. 5.

¹H-NMR spectra acquired at 800 MHz and assignment of heparin, contaminated heparin and OSCS contaminant. **A.** ¹H-NMR spectrum of heparin. a, H1 GlcNS, GlcNS6S; b, H1 IdoA2S; c, H1 IdoA; d, H5 IdoA2S; e, H1 GlcA; f, H6 GlcNS6S; g, H2 IdoA2S; h, H6⁰ GlcNS6S; i, H3 IdoA2S; j, H4 IdoA2S; k, H5 GlcNS5S; l, H6 GlcNS; m, H4 GlcNS6S; n, H3 GlcNS, GlcNS6S; o, H2 GlcA; p, H2 GlcNS6S; q, acetyl CH₃. **B.** ¹H-NMR spectrum of contaminated heparin; peaks with red arrows show peaks not observed in heparin. **C.** ¹H-NMR spectrum of OSCS contaminant. r, H4 GalNAc2S4S6S; s, H3 GlcA2S3S; t, H1 GlcA2S3S; u, H1 GalNAc2S4S6S; v, H2 and 4 of GlcA2S3S; w, H6 GalNAc2S4S6S; x, H5 GlcA2S3S; y, H2,3 and 5 GalNAc2S4S6S; and z, acetyl CH₃ of GalNAc2S4S6S.

Fig. 6.
Contaminant stability in processes used in preparing LMWHs.

Fig. 7.
Current understanding of the biological activities associated with OSCS acute toxicity.

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Fig. 9.
Heparin and heparan sulfate biosynthesis.

Fig. 10.

The stepwise chemoenzymatic synthesis of an anticoagulant heparin.

Table 1Structural variability of heparins between different tissues^{4,5,27}

Tissue	Average number in a single heparin chain			
	<i>N</i> -Acetyl ATIII binding sites	<i>N</i> -Sulfo ATIII binding sites	Trisulfated disaccharides	Disulfated disaccharides
Porcine intestine	0.5 (0.3–0.7) ^a	0.1	10 (10–15)	1.2 (1–2)
Bovine lung	0.3	0.3	14	1.0
Bovine intestine	0.3	0.3	10	1.7
Ovine intestine	0.7	0.4	11	1.4

^aThe numbers shown in parentheses indicate a range of values typically observed.

Table 2**Heparinoids as potential contaminants in heparin**

Heparinoids	Source
Chondroitin sulfate A (CS-A)	Animal tissues
Dermatan sulfate (DS, CS-B)	Animal tissues
Chondroitin sulfate C (CS-C)	Animal tissues
Chondroitin sulfate D (CS-D)	Animal tissues
Hyaluronic acid (HA)	Animal tissues and bacteria
Heparan sulfate (HS)	Animal tissues
Heparosan	<i>E. coli</i> K5
Chitosan sulfate	Semi-synthesis
Dermatan disulfate (DS-diS)	Semi-synthesis
Oversulfated HA (OSHA)	Semi-synthesis
Oversulfated DS (OSDS)	Semi-synthesis
Oversulfated CS (OSCS)	Semi-synthesis
Dextran sulfate	Semi-synthesis
κ-Carrageenan	Seaweed
Fucoidan	Seaweed
Pentosan sulfate	Semi-synthesis
PII88	Semi-synthesis
Poly(vinyl sulfate)	Synthesis
Polyanetholesulfonic	Synthesis
Sucrose octasulfate (SOS)	Semi-synthesis