

Kinetics of Intravenously Administered Heparin in Normal Humans

By Cees A. M. de Swart, Bertha Nijmeyer, Jan M. M. Roelofs, and Jan J. Sixma

Heparin of five commercially available brands was used to study the disappearance of heparin anticoagulant activity in normal humans. The drug was administered intravenously by bolus injection and by continuous infusion. Heparin anticoagulant activity was determined by two assays: a diluted activated partial thromboplastin time (APTT) and an assay based on inactivation of bovine factor Xa, using a clotting system. After a bolus injection, the data fitted neither single exponential nor zero-order clearance. In semilogarithmic plots, heparin anticoagulant activity disappeared according to a slightly convex curve almost always preceded by a rapid initial loss of heparin anticoagulant activity. This disappearance profile was observed with all

heparin regardless of the brand or assay system. Heparin anticoagulant activity estimated by the APTT disappeared faster than heparin anticoagulant activity estimated by the anti-Xa activity in the first phase. As expected, higher anticoagulant levels with the anti-Xa assay than with the APTT were also found on continuous infusion in normals as well as in patients treated for deep vein thrombosis or pulmonary embolism. The experimental data suggested a model based on the combination of a saturable and a linear clearance mechanism. These experimental data provide reliable guidelines for adjustment of the dose of heparin in single patients.

THE LEVEL OF anticoagulation at a given dose of heparin may vary strongly from patient to patient. This is particularly important when heparin is given for an existing thrombosis, where this may cause bleeding or embolization. Various dosage schedules for safe and effective treatment have been advocated,¹⁻¹⁰ but they are not based on firm knowledge of the pharmacokinetics of the anticoagulant effect of heparin.

The anticoagulant activity of intravenously administered heparin has been described as disappearing following a single exponential curve.¹¹⁻¹⁵ It was noted, however, that the half-life increased with the dosage.^{11,12,14}

Recently, new tests have been developed^{16,17} allowing more accurate observation of the kinetics of the anticoagulant effect of heparin. We report the application of these tests to the study of the kinetics of the anticoagulant effect of five commercially available brands of heparin.

MATERIALS AND METHODS

Subjects

Subjects were male and female volunteers: students, physicians, and biochemists of the Department of Internal Medicine and the Department of Hematology. Female volunteers who were menstruating and volunteers of either sex presenting with a history of hypertension, a previous gastroduodenal ulcer disease, or any hemorrhagic tendency were excluded from the study.

Ages of the volunteers ranged from 21 to 40 yr. The investigation was approved by the Committee on Medical Ethics of our hospital. The nature, purpose, and possible risks of the study were fully explained to each subject before obtaining voluntary consent.

Heparin

Five commercially available brands of heparin were employed in the study. The heparin preparations were obtained from Leo Pharmaceutische Produkten, Emmen, The Netherlands; Organon, Oss, The Netherlands; Novo Industries A/S, Copenhagen, Denmark; Roussel Uclaf, Paris, France; and the Upjohn Company, Kalamazoo, Mich.

Details concerning these preparations are shown in Table I.

Chemicals

The activated partial thromboplastin time reagent was from Boehringer GmbH, Mannheim, West Germany. Bovine factor Xa, cephalin, and anticoagulant-free bovine plasma were from Sigma Chemical Co., St. Louis, Mo. Bovine factor Xa (Diagen) was from Diagnostic Reagents, Thame, Oxon, England.

Blood Collection and Storage

Blood was collected from an indwelling catheter (16-gauge diameter) positioned in a forearm vein. After discarding the first milliliter, 9 volumes of blood were collected in polystyrene tubes containing 1 volume of 0.129 M trisodium citrate and centrifuged (10 min, 3500 g, 4°C). The supernatant platelet-poor plasma was stored frozen at -70°C until assayed. The determinations of the heparin anticoagulant activities were performed within 1 wk after blood collection. This storage interval did not influence the anticoagulant activity of the plasma samples, as separately tested.

Normal Plasma

Normal plasma, derived from 40 healthy hospital staff volunteers (ratio women/men 1:1, nonpregnant women who were not taking oral contraceptives), was pooled and stored at -70°C in 1-ml aliquots.

Assays of Heparin Anticoagulant Activity

Two assays were employed in the study.

(1) A diluted activated partial thromboplastin time (APTT) was performed according to Marder¹⁶ with slight modifications. The heparin-containing test plasma was diluted (1:4, 1:10, 1:20, or 1:40) with normal plasma, depending on the expected heparin anticoagulant activity.

From the Department of Haematology and Department of Medical Physics, State University Hospital, Utrecht, The Netherlands.

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Address reprint requests to J. J. Sixma, M.D., Department of Hematology, State University Hospital, Catharijnesingel 101, Utrecht, The Netherlands.

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Table 1. Properties of Heparin Preparations

Heparin Brand	Origin	Lot Number	Anticoagulant Potency (U/mg)	Number of Experiments	
				APTT	Anti-Xa Assay
Leo	Porcine mucosal	65217111, 77347111, 89307111, 89737111, 71628111, 91898111, 71009111, 77219111	160-175*	12	6
Upjohn	Beef lung	350 DH	139.2†	2	2
Roussel	Porcine mucosal	6 S 0405	168†	1	1
Novo	Porcine mucosal	Q 449	154†	2	2
Organon	Porcine mucosal	726335	167*	2	2

It should be noted that some commercial preparations may have been obtained from the same manufacturer of crude material.

*According to the Third International standard.

†USP units.

lant activity. A quantity of 0.1 ml of diluted test plasma and 0.1 ml of a mixture of equal parts of kaolin and cephalin was incubated for 3 min at 37°C. Of 0.025 M CaCl₂, 0.1 ml was added and the clotting time was recorded with a Kolle hook. All determinations were performed in triplicate. Clotting times were converted into heparin anticoagulant activities with the aid of a calibration curve, previously constructed by addition of 0.1-ml aliquots of various heparin concentrations of the same heparin brand to 0.9 ml of normal plasma. The mixture was further treated as described above. The variation coefficient of the assay was 5% in the range between 0.25 and 5.0 U/ml and 12% at 0.1 U/ml. The assay was unreliable below 0.1 U/ml.

(2) An anti-Xa assay, using a clotting system, was performed as described by Yin¹⁷ with slight modifications. Heparin-containing test plasma was diluted (1:20, 1:10, 1:5, or undiluted) with normal plasma, depending on the expected heparin anticoagulant activity. A quantity of 0.2 ml of bovine factor Xa (3-4 U/ml) was added to 0.2 ml of diluted test plasma and incubated for 90 sec at 37°C. Of the resultant mixture, 0.1 ml was transferred to another tube at 37°C. After 20 sec, 0.1 ml of 0.025 M CaCl₂ was added and after another 10 sec, 0.2 ml of a mixture of equal parts of cephalin and anticoagulant-free bovine plasma was added. The clotting time was recorded. The test was performed in duplicate. Clotting times were converted into heparin anticoagulant activities by means of a calibration curve, constructed as described above. The variation coefficient of the assay was 10% over the whole range of 0.01-5.0 U/ml. Linear regression curves of observed heparin anticoagulant activities as measured by the two assays versus expected heparin anticoagulant activities, when normal plasma had been heparinized in vitro, covering the range from 0 to 5.0 U heparin/ml plasma, are presented in Fig. 1. Slopes and correlation coefficients are near 1.0.

Single Dose Experiments

Two-hundred and fifty units of heparin/kg body weight were administered intravenously. Blood samples were collected at 2, 5, 10, 15, 30, 45, and 60 min after injection and subsequently at 30-min intervals until 540 min after injection.

Continuous Infusion Experiments

Heparin was added to sterile 0.154 M NaCl in a syringe. The syringe was placed on a continuous infusion pump (Sage Instruments Cambridge, Mass.), preset to deliver a fixed volume of fluid. The infusion pump was checked for delivering the indicated volumes of fluid. Evidence was obtained that the heparin anticoagulant activity of a heparin solution in a syringe did not deteriorate during the infusion period (data not shown). Continuous infusion for 18 hr was performed in 3 normal individuals, who were confined to bed for

the duration of the infusion. The dosage was changed every 6 hr. Samples for estimation of heparin anticoagulation levels were taken at an interval of 30 min. Heparin anticoagulant activities were also assayed in 6 patients on continuous heparin therapy for deep vein thrombosis or pulmonary embolism. Samples for estimation of heparin levels were taken at 24-hr intervals.

Mathematical Procedures

For reasons that will be explained under Results, we attempted to fit the data to two models: Model 1: A combination of a saturable and a first-order elimination mechanism in parallel. Model 2: A single saturable elimination mechanism subjected to product inhibition, assuming first-order kinetics for product elimination.

The mathematical expression for the first model is:

$$\frac{dC}{dt} = -LC - \frac{V_m C}{C + K_m} + I$$

The second model can be expressed as two coupled equations:

$$\frac{dC}{dt} = - \frac{V_m C}{C + K_m \left(1 + \frac{P}{K_p}\right)} + I$$

$$\frac{dP}{dt} = \frac{V_m C}{C + K_m \left(1 + \frac{P}{K_p}\right)} - K_e P$$

In the equations dC/dt stands for the elimination rate of heparin anticoagulant activity, L stands for the slope of the first-order elimination mechanism, V_m for the maximal elimination of the saturable mechanism, K_m for the Michaelis-Menten constant of the saturable mechanism, I for the heparin infusion rate (for injections, I represents a pulse function), P for the concentration of a hypothetical metabolic heparin product that is supposed to have an inhibitory effect on heparin anticoagulant activity, K_p for the inhibition constant of the product, dP/dt for the elimination rate of the product, and K_e stands for the slope of a postulated first-order elimination mechanism of the product.

The data of the single-dose experiments were fitted to the well known integrated form of the first equation.¹⁸ Optimization was performed by a least-squares method. The sensitivity of the function to changes in the parameters was evaluated in a program by determining the matrix of the second derivatives of the sum to the parameter vector.¹⁹

Fitting of the single-dose experimental data to the second model was performed using the NLFIT program. This program is available on request at Acculib, Instituut voor Mathematische statistiek, Budapestlaan 6, de Uithof, Utrecht, The Netherlands.

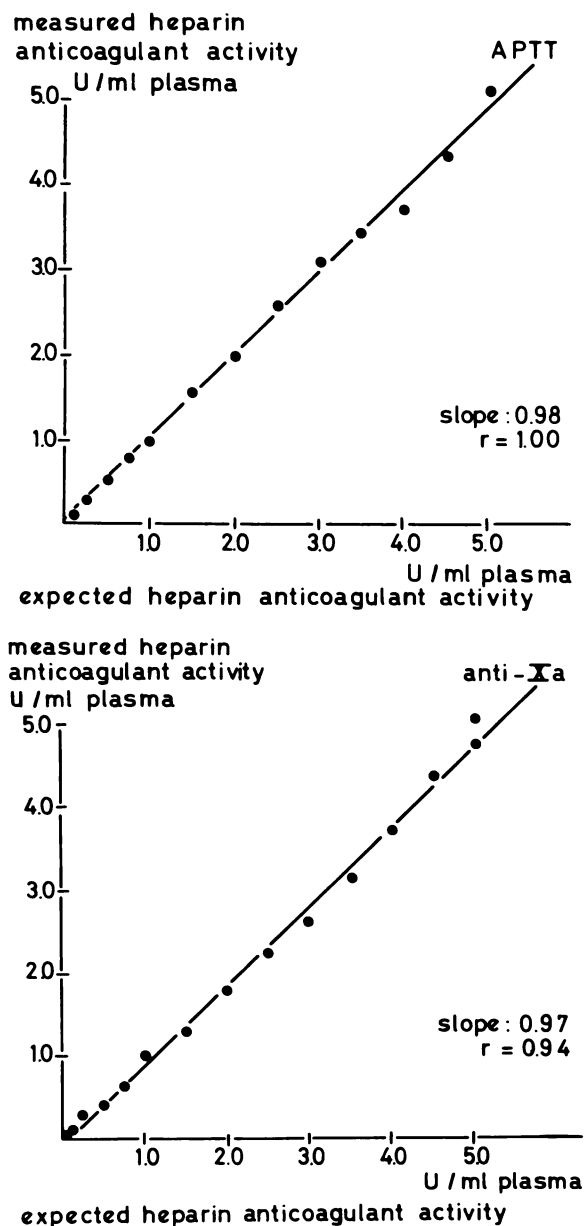


Fig. 1. Relationship between heparin anticoagulant activities expected after addition of heparin to normal plasma and in vitro heparin anticoagulant activities measured by APTT (A) and the anti-Xa assay (B). Each point represents the mean of five independent determinations.

The parameters were estimated by an optimization program in which the function values were obtained by numerical integration according to the Runge Kutta method. Optimization was performed by a least-squares method. For the computations, a Hewlett-Packard computer, type HP 9830, and a CDC Cyber 73-28 computer were used.

RESULTS

Table 1 summarizes the characteristics of the heparin preparations, the number of experiments performed, and the assays that we have used.

Single-Dose Experiments

A representative disappearance curve of heparin anticoagulant activity is shown in Fig. 2. For reasons of comparisons, the curve is plotted on a linear (Fig. 2, A and C) and on a semilogarithmic scale (Fig. 2, B and D). Obviously, heparin anticoagulant activity disappears neither according to first- nor a zero-order elimination process. The semilogarithmically plotted curve can be described by a rapid initial disappearance phase, followed by a convex curve. Such an elimination pattern was observed with all heparins, regardless of the brand, or the assay system. The rapid initial disappearance phase lasted 10–15 min. The recoveries of heparin anticoagulant activity at zero-time were near 100% when the entire curve was fitted by eye. For this purpose, the plasma volume was calculated with

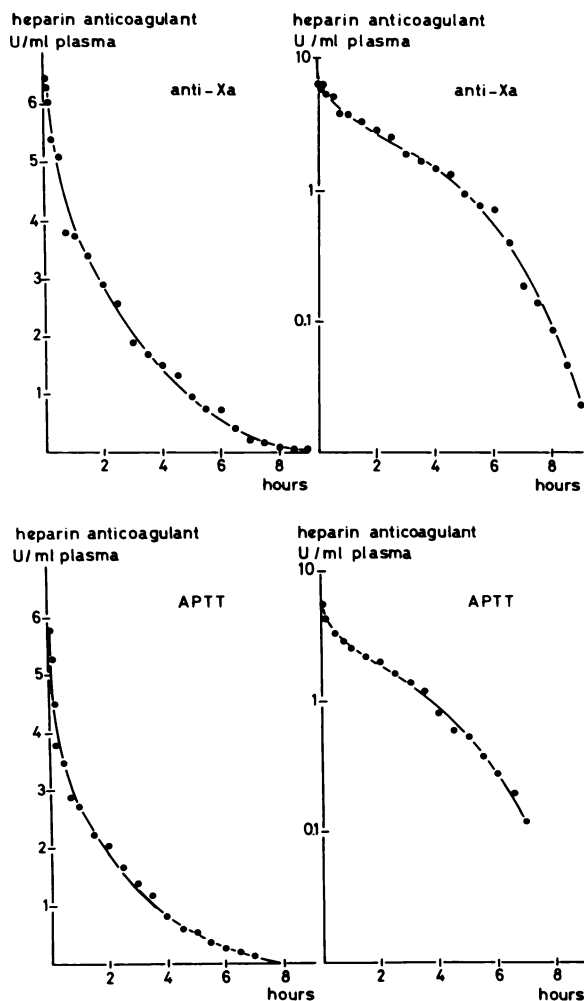


Fig. 2. Linearly (A, C) and semilogarithmically (B, D) plotted elimination curve of heparin anticoagulant activity as measured by the anti-Xa assay (A, B) and APTT (C, D); 250 U of heparin/kg body weight was administered.

the help of the formula: blood volume (in liters) = $2.7 \times$ body surface area (in sq m) - 0.14.

The simultaneous use of APTT and anti-Xa assays allowed a comparison of the two assays in determining heparin anticoagulant activity in vivo. Heparin anticoagulant activity, as estimated by the APTT, disappeared faster shortly after administration than heparin anticoagulant activity estimated by the anti-Xa assay. In the later part of this disappearance curve, the ratio between the two activities remained constant. A mean ratio anti-Xa/APTT of 1.45 ± 0.09 (SEM) was observed (Fig. 3). A similar ratio was also found when the data from 6 patients on continuous heparin infusion for treatment of deep vein thrombosis or pulmonary embolism were plotted. Combination of the data in a single plot was allowed because no clustering of data of a single patient was observed (Fig. 4).

Continuous Infusion Experiments

A representative example of continuous infusion at 3 dosage levels is shown in Fig. 5. The level of anticoagulant activity assayed with the anti-Xa assay was consistently higher than that measured with the APTT. A plateau level was reached at the dosage of 10,000 U/24 hr and for the APTT at 20,000 U/24 hr. A

anti Xa assay
U/ml plasma

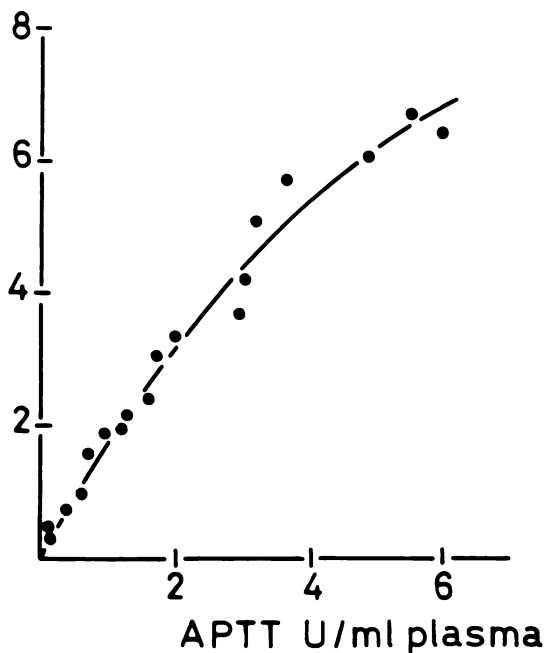


Fig. 3. Relationship between heparin anticoagulant activity estimated with the APTT and with the anti-Xa assay in vivo after a single bolus injection of 250 U/kg. The slope is calculated below 3.8 U anti-Xa/ml.

anti Xa assay
U/ml plasma

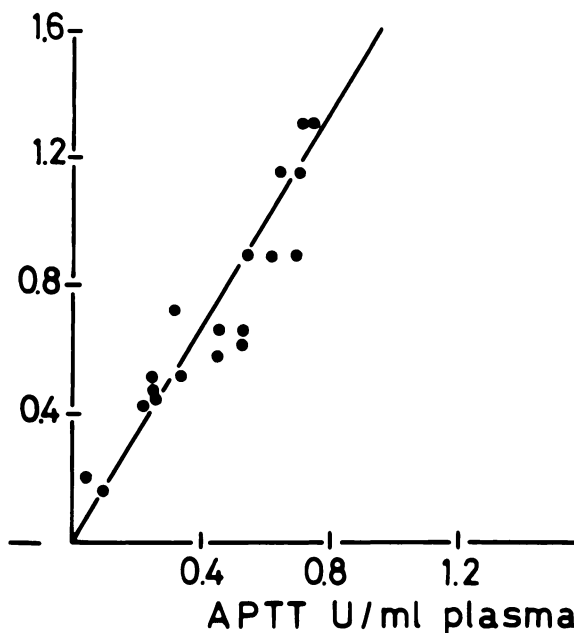


Fig. 4. Relationship between heparin anticoagulant activity estimated with the APTT and with the anti-Xa assay in patients on continuous heparin therapy for thromboembolic disease. The figure contains data from 6 different patients. These data could be combined because no evidence of clustering was seen. Please note the difference in scale between A and B.

plateau level was not quite reached for the anti-Xa values at 20,000 U/24 hr and at 30,000 U/24 hr for either of the two activities.

Curve Fitting

Curve fitting was attempted in order to obtain a model that may describe the kinetics of the heparin anticoagulant activity. Two models are generally pro-

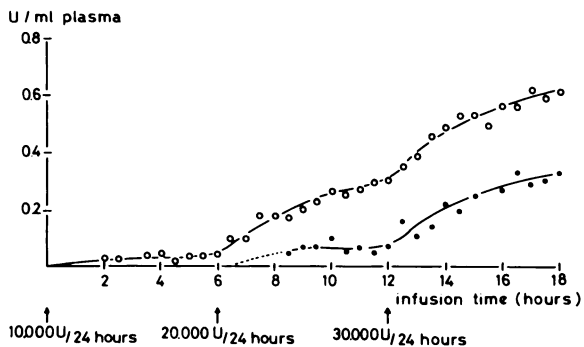


Fig. 5. Relationship between measured heparin anticoagulant activities and heparin infusion rates observed in a continuous infusion experiment. Heparin anticoagulant activity was estimated by an anti-Xa assay (○—○) and APTT (●—●).

posed for nonlinear kinetics of this nature.²⁰ The combination of a saturable and first-order mechanism is the most simple model to describe these data (for curve-fitting procedures, see Materials and Methods). The integrated form of the equation of model 1 fitted well to the single-dose experiments, but an additional initial equilibration phase had to be assumed to explain the rapid disappearance phase. Enough data are available to suggest that such rapid initial removal does indeed occur.^{11,15} The continuous infusion data (Fig. 5) fitted well to this model.

An attractive alternative is the model in which product inhibition is operative. This model was suggested to us because similar curves to those observed after a single dose were obtained by computer simulation studies in which the effects of product inhibition on a simple Michaelis-Menten model were explored.²³

As this would describe the entire curve in terms of a biologically meaningful model, we also tested this alternative. The continuous infusion curves found experimentally appeared also to be predicted by this model, although less well than the combination of a saturable and first-order elimination mechanism.

Two additional sets of experiments were performed to investigate the plausibility of the product inhibition model. This model predicts that the elimination rate after larger single doses will decrease proportionally to the amount of "product" causing divergent elimination curves. The elimination curves observed were more or less parallel, however, (Fig. 6) in agreement with the description of the data given by the first model.

We also tested the product inhibition model by administering a single dose of heparin followed by neutralization of heparin anticoagulant activity with protamine chloride. This should cause accumulation of the postulated inhibitory product, provided that this product would not lose its inhibitor property by possible binding to protamine chloride itself. A second dose was then administered, but the elimination of the anticoagulant activity was similar to that after the first dose (Fig. 7), which makes the existence of an inhibitory product less likely.

DISCUSSION

After intravenous administration, heparin anticoagulant activity has been reported to disappear exponentially in dogs¹¹ and man.¹¹⁻¹³ Other investigators confirmed these first-order kinetics,^{14,15} except Simon, who described the elimination of heparin anticoagulant activity as combined zero- and first-order kinetics.²⁴ Some authors noticed a slightly faster initial disappearance,^{11,15} which was interpreted as a distribution phenomenon. Most investigators agreed that hep-

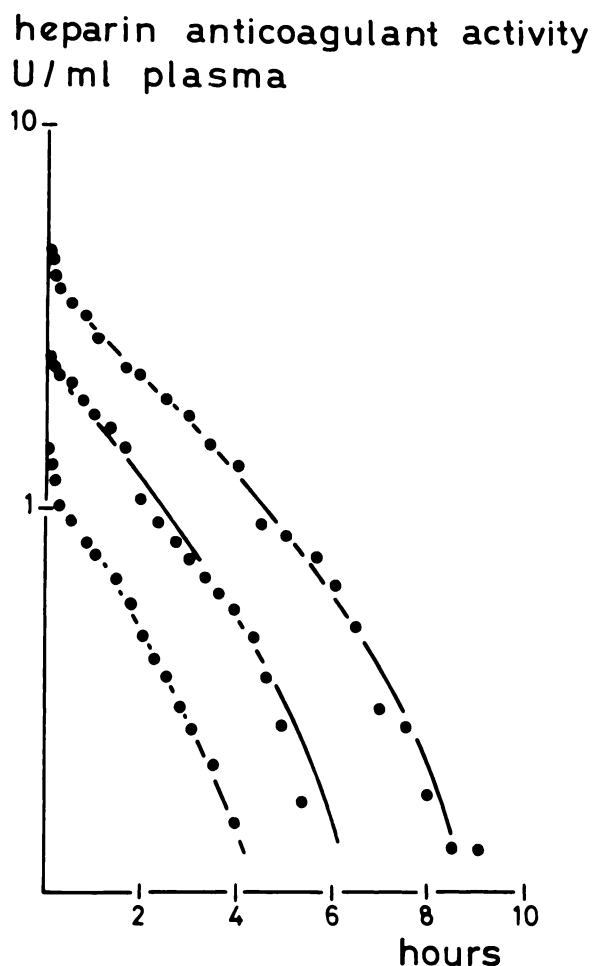


Fig. 6. Parallel elimination curves of heparin anticoagulant activity (APTT) after intravenous administration of 75, 150, and 250 U of heparin/kg body weight. The doses were administered on 3 consecutive days.

arin half-life increased with dosage when administered as a bolus,^{11,12,14} although the observed first-order kinetics dictate a constant half-life.

In an attempt to explain this paradoxical finding we have studied the disappearance of heparin anticoagulant activity after intravenous injection and during continuous infusion. In order to detect small departures from first-order kinetics, decline of heparin anticoagulant activity was followed after a single relatively high dose until low levels of anticoagulant activity were achieved.

In the single-dose experiments, we observed almost always a fast initial removal of heparin anticoagulant activity followed by a slightly convex curve, when the logarithm of the heparin anticoagulant activity was plotted as a function of time. The rapid initial decay of heparin anticoagulant activity suggested an equilibration phase, which was interpreted to result from mixing

heparin anticoagulant activity U/ml plasma

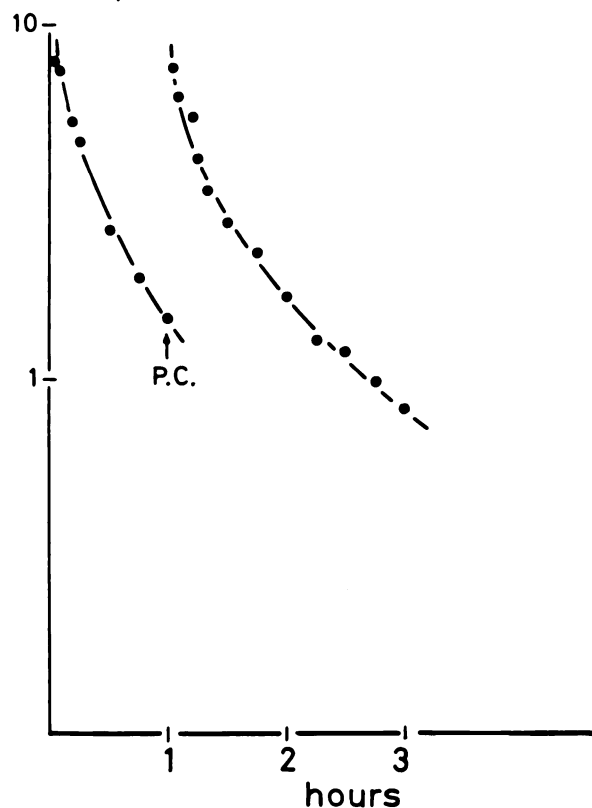


Fig. 7. Two-hundred and fifty units of heparin/kg body weight were administered intravenously. Remaining heparin anticoagulant activity in vivo after 60 min was neutralized by protamine chloride (P.C.) and was followed immediately by a second dose of heparin of similar size. The elimination curves are parallel.

of the drug in plasma and from distribution to another compartment. The last interpretation agrees well with observations of Glimelius²¹ and Mahadoo,²² who showed a considerable binding of heparin to endothelium.

The mentioned convex disappearance pattern preceded by a fast initial decline was seen with all heparins regardless of the brand or assay system. The convex part of the curve offers a satisfactory explanation for the dose-dependent half-life, as has been reported previously. Probably, tangents to a slightly convex curve line have been drawn by previous authors. A plausible explanation why we observed a nonlinear elimination pattern is the large number of measurements until low levels of heparin anticoagulant activity were attained.

The disappearance of heparin anticoagulant activity was followed by both the APTT and by an assay based on the factor Xa inhibiting property of the heparin-antithrombin-III complex. This allowed a comparison

of the two methods. The relationships between values found with the APTT and the anti-Xa assay during single-dose experiments was not linear, but could be described by a convex curve above 2.0 U/ml and a linear part with a slope of 1.45 below 2.0 U/ml when the anti-Xa activities were plotted as a function of the APTT activities. This was in contrast to the linear relationship with a slope of 1.0, which was obtained when these methods were compared in determining heparin anticoagulant activities of normal plasma after *in vitro* addition of heparin comprising the same range of heparin anticoagulant activities (Fig. 1). Apparently, *in vivo*, the heparin anticoagulant activity estimated by the APTT disappears faster than the anticoagulant activity estimated by an assay based on factor Xa inactivation shortly after heparin injection. The observation predicts that during continuous infusion of heparin, activity measured with the anti-Xa assay will be higher than that measured with the APTT. Experimentally this was indeed observed in patients (Fig. 4) as well as in normals (Fig. 5).

Recently, purified fractions with different molecular weights have been prepared from crude commercial heparin by affinity chromatography on antithrombin-III-Sepharose and subsequent gel chromatography.²⁵

The observed overall disappearance curve of the anticoagulant activity of crude heparin might result from the different disappearances of various subfractions of this heterogeneous material. Data (to be published elsewhere) obtained with subfractions have shown that this is not so. Almost all fractions disappear similarly to the crude material, with the exception of some low molecular weight material that possessed almost exclusively anti-Xa activity.

Although the elimination of most drugs from the plasma permits the application of a model with one or more exponentials, definite, nonlinear kinetics have been described in the literature for several drugs, e.g., acetyl salicylic acid²⁶ and diphenyl hydantoin.^{27,28} Involvement of saturable processes such as metabolic pathways in the liver and renal tubular transport processes may account for nonlinear kinetics. This has been elegantly shown by Levy et al., who investigated in detail the kinetics of acetyl salicylic acid.²⁶

Our single-dose elimination curves did not allow us to adopt a simple linear kinetic model. Continuous heparin infusion experiments confirmed this nonlinearity and also suggested that the observed data could be described most easily by a saturable and a linear removing elimination mechanism in parallel. Basic information about physiologic and anatomic data should be incorporated in the construction of a meaningful kinetic model. Some knowledge about heparin metabolism is available. Dawes and Pepper²⁹ charac-

terized heparin in plasma after injection of an iodinated heparin derivative. They demonstrated that heparin was degraded by desulphation, thereby losing its anticoagulant activity. Early reports pointed to a role of the mononuclear phagocytes in the uptake and desulphation of heparin.^{30,31} This role was substantiated by the isolation of a heparin desulphamidase from lymphoid tissue of rat, dog, and man^{32,33} and by the observation that cultured mouse peritoneal macrophages can desulphate heparin.³⁴ Desulphating enzymes may well fit into the concept of a saturable elimination mechanism as part of our elimination model.

The physical and biologic properties of heparin excreted in urine are dependent on the dose of heparin administered.^{2,35-38} After intravenous administration of small doses (10–5000 U) of heparin, the drug is excreted in urine as more or less desulphated molecules,²⁹ part of them smaller than the injected heparin molecules, possibly by the action of a postulated readily saturated endoglycosidase in the kidney. Partly desulphated heparin molecules, designated as uroheparin by McAllister,³⁷ retain only 50% of their original anticoagulant activity. After larger doses of heparin, intact (not desulphated) heparin molecules with full preservation of their anticoagulant activity are excreted in urine. The elimination of unmodified heparin at larger doses may represent the second component of our elimination model, namely the mechanism that removes heparin anticoagulant activity linearly. This type of elimination is probably not explained by glomerular filtration, since the negatively charged heparin molecules will be repelled by negatively charged heparin sulphate molecules abundantly present in the basement membrane of the renal glomeruli.³⁹

The disappearance of the anticoagulant activity of heparin follows nonlinear kinetics and can be described by a combination of a saturable and a linear mechanism. This has important consequences for the adjustment of the dosage of heparin in patients to whom heparin is administered for an existing thrombosis. Simulation of continuous infusions with parameters obtained from bolus injection experiments show that there is a sort of threshold below which heparin levels rise only slowly and above which they rise more rapidly linearly correlated to the dose. Figure 8 gives an example of such a simulation. At the right, the relationship between the dosage of heparin (X axis) and the plateau level of heparin (Y axis) is shown. At the left, 3 curves of continuous infusion are depicted in order to illustrate how similar these curves are to what was experimentally observed in Fig. 5.

In clinical practice this means that most patients

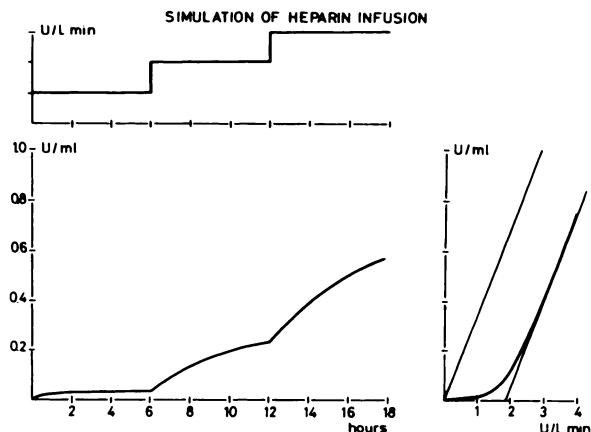


Fig. 8. Simulation of continuous infusion with the use of parameters from a bolus injection experiment using the model of a combined saturable and linear elimination mechanism. The figure at the right shows the relationship between dose of administered heparin and the plateau level. The figure at the left shows the time-dependent change at three chosen rates of heparin administration.

will have almost negligible heparin levels at doses of below 25,000 U/24 hr, whereas the level rises steeply at higher doses. Another consequence is that the time required to attain a plateau level is much prolonged at higher dose levels. This is already evident from the fact that the apparent half-life increases with the increase in heparin level, but it is illustrated in Fig. 8 and Fig. 5. Awareness of these phenomena is of great importance for safe adjustment of heparin therapy. The individual reaction of patients varies widely, and the adequate dose for a single patient has to be found by trial and error. We have found that it pays off to start with a relatively high dose (40,000 U/24 hr) that will give a heparin level in the part of the curve where the first-order removal mechanism is operative and then to adjust the dose to attain the desired level. For administration to patients with deep vein thrombosis or pulmonary embolism, we use an initial bolus injection of 2500 U and we perform the first assay of the heparin anticoagulant level after 4 hr. A plateau level is not yet reached then, but this value allows adjustment of the dose.

The experimental findings also explain why low-dose heparin therapy is producing such low heparin levels. They also predict that an occasional patient in whom the saturable system has a low maximal velocity, may get relatively high heparin levels on such therapy.

These data also have consequences for intermittent therapy. The relatively slower disappearance of heparin anticoagulant activity at high heparin levels will cause accumulation of heparin when the dosage scheme is such that not all heparin has disappeared

before the following injection. This may then lead to such high levels that bleeding may occur.

NOTE ADDED IN PROOF

After this paper had been submitted, a paper came to our attention (T. J. McAvoy, *Journal of Pharmacokinetics and Biopharmaceutics* 7:331-354, 1979) which proposed models for nonlinear kinetics of hepa-

rin closely related to ours on a theoretical basis, suggesting experiments as reported in this paper.

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