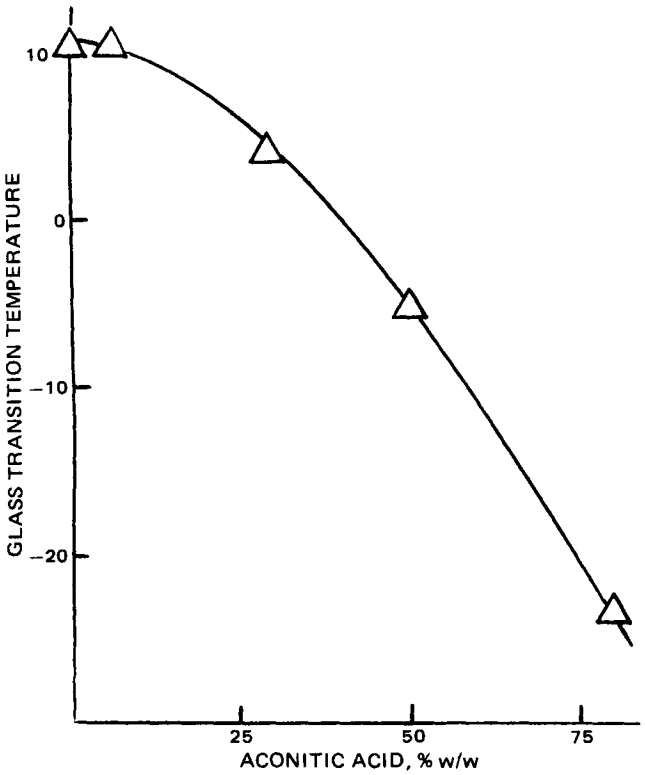


Figure 1—*The effect of aconitic acid on the transition temperature of citric acid glass.*

on prolonged exposure of molten citric acid to temperatures above its melting temperature. It has been shown previously that the sample preparation techniques used in this study did not adversely affect citric acid (12). In addition, the data also imply that low levels of impurities (<5% w/w) cannot be readily detected by DSC.

In preparation of citric acid glass, care should be taken to use the minimum amount of heat necessary to melt the citric acid, to avoid prolonged heating at temperatures of its melting point, and to employ a procedure in which there is some means of temperature control. Although these suggestions for the preparation of citric acid glass may make this material unsuitable for use in the commercial preparation of solid dispersion systems, it should not eliminate the use of this glassy vehicle as a tool for the examination of the compatability, miscibility, and stabilization of the glassy states of materials.

(1) P. Chaudhari and D. Turnbull, *Science*, **199**, 11 (1978).
(2) P. Chaudhari, B. C. Giessen, and D. Turnbull, *Sci. Am.*, **242**, 98 (1980).
(3) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **60**, 1281 (1971).
(4) B. R. Hajratwala, *Aust. J. Pharm. Sci.*, **NS3**, 101 (1974).
(5) J. W. McGinity, *Pharm. Tech.*, **2**, 48 (1978).
(6) W. L. Chiou and S. Riegelman, *J. Pharm. Sci.*, **58**, 1505 (1969).
(7) M. P. Summers and R. P. Enever, *J. Pharm. Pharmacol.*, **26**, 83P (1974).
(8) M. P. Summers and R. P. Enever, *J. Pharm. Sci.*, **65**, 1613 (1976).
(9) *Ibid.*, **66**, 825 (1977).
(10) M. P. Summers, *ibid.*, **67**, 1606 (1978).
(11) J. W. McGinity, D. D. Maness, and G. J. Yakatan, *Drug Dev. Commun.*, **1**, 369 (1974–1975).
(12) R. J. Timko and N. G. Lordi, *J. Pharm. Sci.*, **68**, 601 (1979).
(13) C. E. Nordman, A. S. Weldon, and A. L. Patterson, *Acta Crystallogr.*, **13**, 418 (1960).
(14) G. S. Parks and H. M. Huffman, *J. Phys. Chem.*, **31**, 1842 (1927).
(15) D. Fox, M. M. Labes, and A. Weissberger, Eds., "Physics and Chemistry of the Organic Solid State," vol. 1, Wiley Interscience, New York, N.Y., 1963.
(16) A. R. Ubbelohde, "Melting and Crystal Structure," Oxford University Press, London, England, 1965.
(17) M. P. Summers and R. P. Enever, *J. Pharm. Sci.*, **69**, 612 (1980).
(18) W. W. Wendlandt and J. A. Hoiberg, *Anal. Chim. Acta*, **28**, 506 (1963).
(19) M. T. Saibova, V. K. Bukins, and E. L. Abromova, *Usb. Khim. Zh.*, **9**, 54 (1965); through *Chem. Abstr.*, **63**, 16197c (1965).

Robert J. Timko *
Nicholas G. Lordi [x]
College of Pharmacy
Rutgers—The State University
Piscataway, NJ 08854

Present address: *Ortho Pharmaceutical Corporation, Raritan, NJ 08869.

Received November 27, 1978.
Accepted for publication June 23, 1982.

Dose-Dependent Decrease in Heparin Elimination

Keyphrases □ Heparin—dose-dependent pharmacokinetics □ Pharmacokinetics—dose-dependent decrease in heparin elimination

To the Editor:

Studies on the pharmacokinetics of heparin have revealed dose-dependent (1–4), time-dependent (5), and assay-dependent (4) characteristics. The mechanisms underlying these characteristics are unknown but are likely to reside in the heterogeneity of heparin. Heparin is a natural mammalian glycosaminoglycan consisting of polymeric constituents arranged linearly, with different chain length and chemical composition and with a molecular weight ranging from 3000 to 45,000 (6–8). Recent studies have demonstrated that the antithrombin-III binding site of heparin, which is necessary for its pharmacological action, appears to reside in an oligosaccharide segment of the molecule that has a specific sequence of four to eight monosaccharides, *i.e.*, iduronic and glucuronic acids and glucosamines, with *N*-sulfate, *O*-sulfate, and *N*-acetyl groups being required at specific sites (9–11). The metabolism of heparin is not well understood. Although the metabolic processes involved are thought to include depolymerization and desulfation, the relationship between different metabolic processes and the decline in anticoagulant activity is unclear.

The biologic half-life of heparin increases with increasing dose in humans and animals (1–4). This dose-dependence is without any indication of Michaelis-Menten type kinetic characteristics (3, 4) and recently has been demonstrated in humans to be due to a dose-dependent decrease in the total clearance of the anticoagulant (4). The total clearance of heparin in humans usually is reported to be between 0.5 and 2 ml/min/kg, while the apparent volume of distribution is usually reported to be between 40 and 100 ml/kg (2, 4, 12–14). However, reported values for both of these pharmacokinetic parameters vary widely

0022-3549/82/01000-1186$01.00/0

among studies. Although this variability in part may be due to the nonlinearity in the elimination kinetics of heparin, it is in part due to differences in heparin assay methods used, since recent studies in humans have shown that there are significant differences among values of individual pharmacokinetic parameters obtained, depending on heparin assay methodology (4). This applies primarily to total clearance and apparent volume of distribution, both of which were on the average ~1.5- to twofold larger when based on hexadimethrine bromide[1] neutralization assay of heparin than when based on bioassays using the coagulation tests activated partial thromboplastin time and thrombin clotting time. However, no significant differences were noted in values of biologic half-life. The immediate practical consequences of the assay-dependent pharmacokinetics of heparin are that when summarizing published pharmacokinetic parameters for heparin, the assay methods have to be specified, and only data obtained by the same heparin assay can be treated together. This becomes particularly important when exploring relationships between dose and individual pharmacokinetic parameters of heparin. Two studies have been published on heparin pharmacokinetics in normal subjects using heparin assays based on thrombin-induced clotting times, with doses ranging from 50 to 400 U/kg (1, 4). Therefore, it was of interest to use data from these two studies to explore relationships between dose and pharmacokinetic parameters of heparin, particularly since one of the studies had not provided values for the latter.

The subjects studied in these two studies were four healthy males, ages 27–35 years, who received intravenous injections of heparin of 50 and 75 U/kg body weight each (4), and six healthy males and seven healthy females, ages 19–45 years, who received intravenous injections of heparin of either 100 (five subjects), 200 (four subjects), or 400 (four subjects) U/kg body weight (1). Multiple blood samples were collected after each dose, and plasma heparin activity was determined by bioassays based on thrombin clotting time. These assays are described in detail elsewhere (4, 15). They involve first establishing the relationship between the thrombin-induced clotting times and heparin added to plasma *in vitro*, and then deriving the plasma heparin activity in the samples obtained after the dose from the observed clotting times. One of these assays uses addition of bovine plasma to the plasma sample (15). While this will attenuate intersubject differences in the relationship between clotting time and added heparin, it will not affect the determination of heparin activity in plasma. It should be noted that the relationship between thrombin-induced clotting times and heparin activity in plasma is log linear (4, 15, 16).

The pharmacokinetic parameters, biologic half-life ($t_{1/2}$), total clearance (Cl), and apparent volume of distribution (V_d), were calculated as described previously (4). As is shown in Fig. 1, the biologic half-life of heparin increases with increasing dose. The average ($\pm SD$) $t_{1/2}$ values for the 50, 75, 100, 200, and 400 U/kg doses were 42 ± 5, 49 ± 15, 57 ± 8, 96 ± 10, and 153 ± 10 min, respectively. The positive relationship between the biologic half-life and dose of heparin is statistically highly significant ($p < 0.001$).

[1] Polybrene.

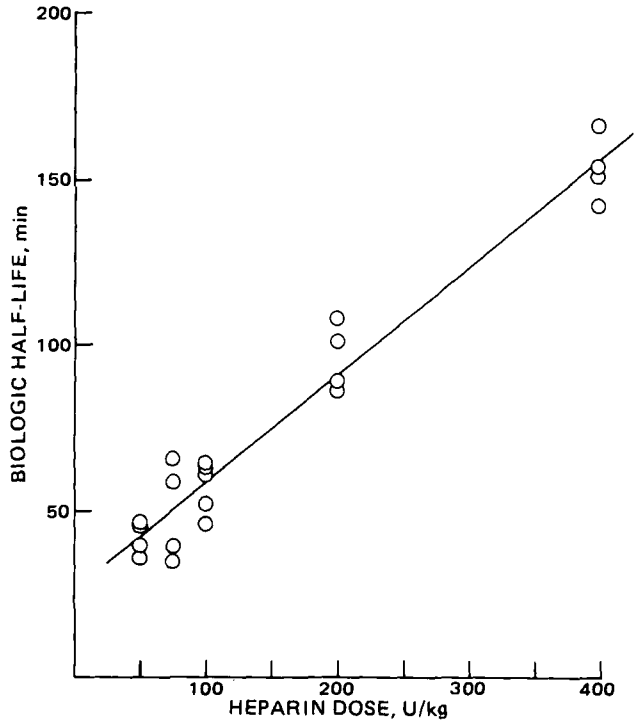


Figure 1—*Relationship between biologic half-life and dose of heparin in humans when heparin activity in plasma is determined by a bioassay based on thrombin-induced clotting times. (Data are from Refs. 1 and 4). The line represents the best-fitted line for the relationship between these parameters (intercept, 26.2 min; slope, 0.323 min kg U^{-1}; r^2 = 0.952).*

Figure 2 shows that there is also a statistically highly significant positive linear relationship between the biologic half-life and total clearance of heparin ($p < 0.001$). The

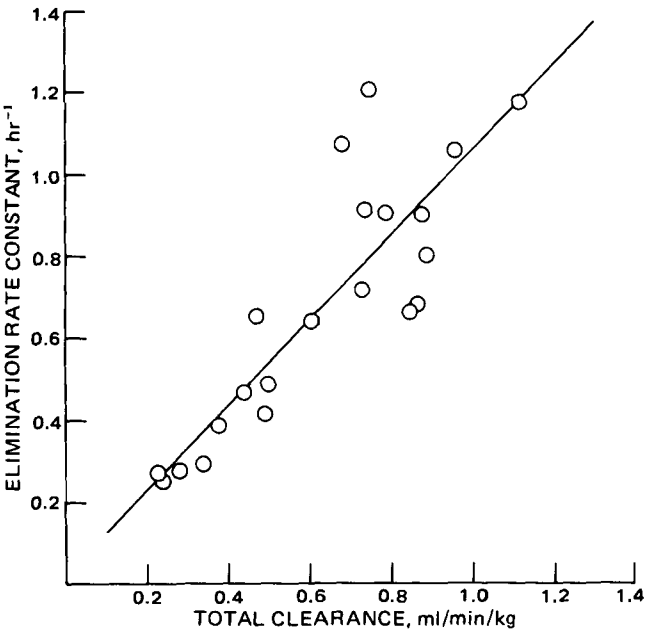


Figure 2—*Relationship between the elimination rate constant and total clearance of heparin in humans when heparin activity in plasma is determined by a bioassay based on thrombin-induced clotting times. (Data are from Refs. 1 and 4.) The line represents the best-fitted line for the relationship between these parameters (intercept, 0.030 hr^{-1}; slope, 1.025 t ml^{-1} kg^{-1}, where t is a time unit correction factor; r^2 = 0.739).*

total clearance of heparin ranged from 0.23 to 1.12 ml/min/kg for the 400 and 50 U/kg doses, respectively, representing about a fivefold variation in total clearance for eightfold difference in dose. The reason for this linear positive relationship is that the apparent volume of distribution of heparin changes only very modestly with dose. The average ($\pm SD$) V_d was 58 ± 11 ml/kg body weight over the entire dose range, with small but statistically insignificant changes in V_d when it was evaluated with respect to dose (intercept, 55 ml/kg; slope, 0.018 ml/kg/U; r^2 = 0.046).

The results presented in this report are in support of and extend recent findings that the dose-dependent increase in the biologic half-life of heparin in humans is due to a dose-dependent decrease in the total clearance of the anticoagulant (4). In humans, there is no significant increase in the apparent volume of distribution of heparin with dose. This is in contrast to findings in rats and dogs, which have shown a dose-dependent increase in V_d with dose (2, 3). While the mechanism underlying the nonlinear pharmacokinetics of heparin in humans is presently not understood, however, the linear relationship between dose and dose-dependent pharmacokinetic parameters is noteworthy.

(1) P. Olsson, H. Lagergren, and S. Ek, *Acta Med. Scand.*, **173**, 619 (1963).

(2) J. W. Estes, E. W. Pelikan, and E. Kruger-Thiemer, *Clin. Pharmacol. Ther.*, **10**, 329 (1969).

(3) T. D. Bjornsson and G. Levy, *J. Pharmacol. Exp. Ther.*, **210**, 237 (1979).

(4) T. D. Bjornsson, K. M. Wolfram, and B. B. Kitchell, *Clin. Pharmacol. Ther.*, **31**, 104 (1982).

(5) T. D. Bjornsson and G. Levy, *J. Pharmacol. Exp. Ther.*, **210**, 243 (1979).

(6) H. B. Nader, H. M. McDuffie, and C. P. Dietrich, *Biochem. Biophys. Res. Commun.*, **57**, 488 (1974).

(7) U. Lindahl and M. Hook, *Annu. Rev. Biochem.*, **47**, 385 (1978).

(8) L. Jaques, *Pharmacol. Rev.*, **31**, 99 (1979).

(9) R. D. Rosenberg, G. M. Oosta, R. E. Jordan, and W. T. Gardner, in "Chemistry and Biology of Heparin," R. L. Lundblad, W. V. Brown, K. G. Mann, and H. R. Roberts, Eds., Elsevier/North Holland, New York, N.Y., 1981, p. 249.

(10) M. Hook, L. Thunberg, J. Riesenfeld, G. Backstrom, I. Pattersson, and U. Lindahl, in "Chemistry and Biology of Heparin," R. L. Lundblad, W. V. Brown, K. G. Mann, and H. R. Roberts, Eds., Elsevier/North Holland, New York, N.Y., 1981, p. 271.

(11) J. Choay, J. C. Lormeau, M. Petitou, P. Sinay, and J. Fareed, *Ann. N.Y. Acad. Sci.*, **370**, 644 (1981).

(12) R. J. Cipolle, R. D. Seifert, B. A. Neilan, D. E. Zaske, and E. Haus, *Clin. Pharmacol. Ther.*, **29**, 387 (1981).

(13) J. Hirsh, W. G. Van Aken, A. S. Gallus, C. T. Dollery, J. F. Cade, and W. L. Yung, *Circulation*, **53**, 691 (1976).

(14) T. L. Simon, T. M. Hyers, J. P. Gaston, and L. A. Harker, *Br. J. Haematol.*, **39**, 111 (1978).

(15) M. Blomback, B. Blomback, P. Olsson, G. William-Olsson, and A. Senning, *Acta Chir. Scand., Suppl.*, **245**, 259 (1959).

(16) T. D. Bjornsson and K. M. Wolfram, *Eur. J. Clin. Pharmacol.*, **21**, 491 (1982).

Thorir D. Bjornsson

Division of Clinical Pharmacology
Departments of Pharmacology and Medicine
Duke University Medical Center
Durham, NC 27710

Received June 1, 1982.

Accepted for publication July 14, 1982.

Supported by Grant No. HL24343 from the National Institutes of Health.

Thorir D. Bjornsson is a Nanaline Duke Scholar and a recipient of a Pharmaceutical Manufacturers Association Foundation Faculty Development Award in Clinical Pharmacology.

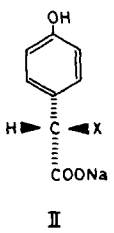
Renal Excretion of *R*- and *S*-Epimers of Moxalactam in Dogs

Keyphrases □ Stereoisomers—arylmalonylamino 1-oxacephem, renal clearance, dog, plasma protein binding □ Renal clearance—arylmalonylamino 1-oxacephem, comparison between stereoisomers, relation to plasma protein binding □ Protein binding—dog plasma, arylmalonylamino 1-oxacephem, comparison between stereoisomers, relation to renal clearance

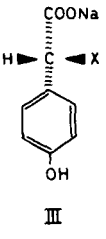
To the Editor:

Moxalactam[1] (latamoxef[2], I) is a mixture of *R* (II) and *S* (III) epimers, and both forms are usually eliminated unchanged by the kidney (1, 2). Studies with humans (3) show that the biological half-life of the *R*-epimer is shorter than that of the *S*-epimer. To obtain a better understanding of the elimination kinetics, we studied the renal clearance and binding of moxalactam epimers to plasma protein in beagle dogs.

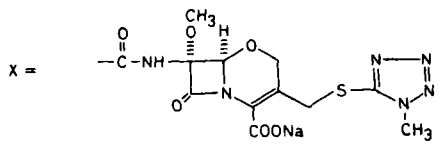
Two male beagle dogs were anesthetized with sodium pentobarbital (30 mg/kg iv). After a tracheotomy was performed, an incision was made in the left flank. The retroperitoneal space was explored and the left ureter was cannulated. Urine was collected through the cannula (4). After completion of the operation, 20 mg of sodium *p*-aminohippurate/kg and 100 mg of creatinine/kg were injected as the priming dose into the axillary vein. As the sustaining dose, a solution containing 15% mannitol, 0.9% NaCl, 0.25% creatinine, and 0.1% sodium *p*-aminohippurate was injected at the rate of 5 ml/min/10 kg. Moxalactam[3] was injected at a priming dose of 10 mg/kg followed by a sustaining dose of 5.0 mg/kg/hr. Approximately 1 hr after beginning the infusion, the urinary output was stabilized at 3–5 ml/min and urine samples were collected three or four times from the left ureter at 3-min intervals. Blood samples were taken at the middle point of each clearance period. The same procedure was repeated in the presence of probenecid (30 mg/kg iv). Collected urine and plasma samples were analyzed for creatinine (5), *p*-ami-



II



III



[1] United States Adopted Name (USAN).
[2] International Nonproprietary Name (INN), 6059-S.
[3] Shionogi & Co., Ltd., Osaka, Japan.

0022-3549/82/01000-1188$01.00/0