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.

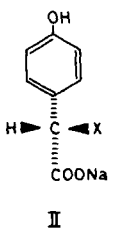
Renal Excretion of *R*- and *S*-Epimers of Moxalactam in Dogs

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

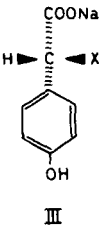
To the Editor:

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.

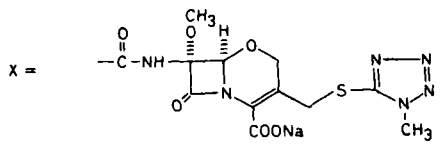
Two male beagle dogs were anesthetized with sodium pentobarbital (30 mg/kg iv). After a tracheotomy was performed, an incision was made in the left flank. The retroperitoneal space was explored and the left ureter was cannulated. Urine was collected through the cannula (4). After completion of the operation, 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

Table I—Renal Clearance (ml/min/kidney) of *R*- and *S*-Epimers of Moxalactam in Anesthetized Male Beagle Dogs

	Dog 1		Dog 2	
	Control phase[a]	Probenecid phase[b]	Control phase[a]	Probenecid phase[a]
Apparent clearances				
R-epimer, total	16.29 ± 0.68[a]	10.81 ± 0.17[b]	17.32 ± 1.18	15.90 ± 0.58
S-epimer, total	9.21 ± 0.52	6.97 ± 0.85[b]	10.48 ± 0.43	10.76 ± 0.25
Clearance ratio *R*/*S*, I	1.81 ± 0.11	1.59 ± 0.19[b]	1.66 ± 0.10	1.48 ± 0.07
Corrected clearances				
R-epimer, 23.7% binding	21.73 ± 0.74	14.19 ± 0.20[b]	22.57 ± 1.55	20.01 ± 0.26
S-epimer, 44.9% binding	16.68 ± 0.95	12.67 ± 1.56[b]	19.03 ± 0.82	19.52 ± 0.44
Clearance ratio *R*/*S*, II	1.32 ± 0.09	1.16 ± 0.14[b]	1.19 ± 0.07	1.07 ± 0.05
Statistical differences[c] of *R*/*S* values between I (total) and II (unbound)	$p < 0.05$	NS	$p < 0.01$	$p < 0.01$
Glomerular filtration rate (Creatinine clearance)	18.16 ± 0.19	14.33 ± 0.88[b]	18.67 ± 0.37	17.04 ± 0.44

[a] Value represents mean ± *SE* (number of samples: $n = 4$). [b] Number of samples: $n = 3$. [c] Student's t test.

nohippuric acid (6), and moxalactam (7). The ratios of *R*- to *S*-epimer in plasma and urine were determined using high-performance liquid chromatography (8). The fractions of the *R*- and *S*-epimers of moxalactam bound to dog plasma were determined at 37° by an ultrafiltration method[4].

The renal clearances of the moxalactam epimers in Table I were calculated by dividing the urinary excretion (micrograms per milliliter) by the total (bound and unbound) concentrations of the epimers. Clearance of the *R*-epimer was 1.4–1.8 times that of the *S*-epimer. The clearance ratios of *R*- and *S*-epimers to creatinine were less than unity. Since only unbound moxalactam would have been available for glomerular filtration, the clearance value based on the total concentration of the epimer in plasma may be underestimated. To determine the actual glomerular filtration, the protein binding of moxalactam epimers was determined at concentrations of 25, 50, and 100 μg/ml of dog plasma. The mean percentage of the bound fraction calculated from these values was 23.7 ± 1.6% *SEM* ($n = 3$) for the *R*-form and 44.9 ± 0.4% ($n = 3$) for the *S*-form.

As indicated in Table I, the data were corrected for the binding of the *R*- and *S*-epimers, *e.g.*, the clearances were calculated by dividing the urinary excretion (micrograms per minute) by the concentrations of the unbound epimers. The calculated renal clearances of the epimers were nearly equal to the glomerular filtration rate (creatinine clearance) and the *R*-epimer/*S*-epimer clearance ratio was closer to unity. When probenecid was given to Dog 1, the *p*-aminohippurate clearance decreased from 55.3 ± 0.6 to 17.0 ± 1.0 ml/min/kidney (mean ± standard error, $n = 3$), but the *R*- or *S*-epimer/creatinine clearance ratio was not affected.

Stereospecific differences in protein binding have been reported for other drugs (9–12) and vary from one animal species to another (3, 13). Our findings indicate that in the dog, both epimers of moxalactam are excreted by glomerular filtration, and the striking difference in renal clearance of the *R*- and *S*-epimers is due mainly to differences in binding to plasma protein.

(1) J. N. Parson, J. M. Romano, and M. E. Levison, *Antimicrob. Agents Chemother.*, **17**, 226 (1980).

(2) J. Shimada, Y. Ueda, T. Yamaji, Y. Abe, and M. Nakamura, in "Current Chemotherapy and Infectious Disease," Proceedings of the 11th ICC and the 19th ICAAC, American Society of Microbiology, 1980, p. 109.

(3) H. Yamada, T. Ichihashi, K. Hirano, and H. Kinoshita, *J. Pharm. Sci.*, **70**, 112 (1981).

(4) T. Higashio, Y. Abe, and K. Yamamoto, *J. Pharmacol. Exp. Ther.*, **207**, 212 (1978).

(5) A. L. Chasson, H. T. Grady, and M. A. Stanly, *Am. J. Clin. Pathol.*, **35**, 83 (1961).

(6) H. W. Smith, N. Finkelstein, L. Aliminosa, B. Crawford, and M. Graber, *J. Clin. Invest.*, **24**, 338 (1945).

(7) T. Yoshida, S. Matsuura, M. Mayama, Y. Kameda, and S. Kuwahara, *Antimicrob. Agents Chemother.*, **17**, 302 (1980).

(8) R. Konaka, K. Kuruma, R. Nishimura, Y. Kimura, and T. Yoshida, *J. Chromatogr.*, **225**, 169 (1981).

(9) W. E. Müller and U. Wollert, *Mol. Pharmacol.*, **11**, 52 (1975).

(10) W. Schmit and E. Jähnchen, *Experientia*, **34**, 1323 (1978).

(11) D. T. Witiak and M. W. Whitehouse, *Biochem. Pharmacol.*, **18**, 971 (1969).

(12) A. Yacobi and G. Levy, *J. Pharmacokinet. Biopharm.*, **5**, 123 (1977).

(13) H. Yamada, Y. Ichihashi, K. Hirano, and H. Kinoshita, *J. Pharm. Sci.*, **70**, 113 (1981).

M. Nakamura[x]
K. Sugeno
R. Konaka
H. Yamada
T. Yoshida
Shionogi Research Laboratories
Shionogi & Co., Ltd.
Fukushima-ku, Osaka 553, Japan

Received May 18, 1981.
Accepted for publication May 12, 1982.
The authors thank Dr. Jingoro Shimada, Jikei University School of Medicine, for his helpful comments and Mr. Tomoji Kawabata for his technical assistance.

[4] Centriflo CF25, Amicon Corp., Lexington, Mass.

Effect of Ascorbic Acid on Renal Excretion of Lead in the Rat

Keyphrases □ Ascorbic acid—effect on renal excretion of lead, rats □ Lead—effect of ascorbic acid on renal excretion, rats □ Renal excretion—effect of ascorbic acid, lead, rats

To the Editor:

Lead poisoning is currently treated by chelation therapy using edetate disodium and dimercaprol, both of which have serious side effects. Few studies have been carried out

0022-3549/82/01000-1189$01.00/0