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Saturation of the tubular excretion of β-lactam antibiotics

J. W. BINS* & H. MATTIE
University Hospital Leiden, Department of Infectious Diseases, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands

1 The saturability of the tubular excretion of cloxacillin, benzylpenicillin and cephradine was investigated.

2 Volunteers received a continuous infusion of one of the antibiotics at increasing infusion rates in order to maintain constant plasma concentrations at three different levels. Blood and urine samples were taken every 30 min. Sufficient urinary flow was ensured by a saline infusion (500 ml h^{-1}).

3 Renal clearance of the antibiotic was calculated for the non-protein bound fraction of the drug.

4 Tubular clearance and tubular excretion rate were estimated by using the renal clearance of the antibiotic minus the glomerular filtration rate; the latter was considered to be equal to creatinine clearance.

5 Data were fitted to a Scatchard transformation and, by nonlinear methods, to a Michaelis–Menten equation.

6 Parameters of saturability, i.e. EC_{50} and maximal tubular excretion rate, were established. The values found for EC_{50} were 7.7, 93.0 and 266 mg l^{-1} for cloxacillin, benzylpenicillin and cephradine, respectively. The values calculated for the maximal tubular excretion rate were 1017, 5535 and 4537 mg h^{-1}, respectively.

Keywords saturation kinetics β-lactam antibiotics

Introduction

Most β-lactam antibiotics are eliminated predominantly by renal tubular excretion (Mandell & Sande, 1980). Since this is an active process it should have a maximal limit. There are indeed indications in the literature that renal clearance decreases with an increase in the plasma concentration of, for instance, benzylpenicillin (Bryner *et al.*, 1948; Pers, 1954), dicloxacillin and cloxacillin (Nauta & Mattie, 1976), piperacillin (Tjandramaga *et al.*, 1978), azlocillin (Bergan & Michalsen, 1979) and mezlocillin (Bergan, 1984).

Most of these studies however were not carried out with the intention of elucidating the tubular excretory process. Since at present some β-lactam antibiotics are given in much higher doses than at the time of their introduction, it is possible that saturability of the excretory process has become more important. Apart from being of theoretical interest this could be of practical consequence for the interpretation of bioavailability studies. The present study was undertaken to establish the quantitative parameters of the tubular excretion of three β-lactam antibiotics.

Methods

Antibiotics

Benzylpenicillin and cephradine (Maxisporin®) were a gift from Gist-brocades N.V. (Delft, The

*Present address: Roussel B.V., Hoevelaken, The Netherlands

Netherlands) and cloxacillin (Orbenin®) was obtained from Beecham Farma B.V. (Amstelveen, The Netherlands).

Volunteers

The Review Committee of the Leiden University Hospital had no objections to this study. Healthy volunteers were obtained by advertising in the university weekly magazine. The aims and risks of the investigation as well as the procedure were explained to all applicants, who subsequently gave their consent in writing. Volunteers were excluded if routine liver and kidney function tests were abnormal and if they had a history of allergy to β-lactam antibiotics.

Experimental procedure

The experimental procedure was aimed at maintaining stable plasma concentrations of the antibiotics at a relatively low, a high and finally a very high plasma level during three consecutive periods of 2.5 h. This was accomplished by giving a bolus injection via a cannula in the cubital vein of one arm, followed by a continuous infusion. The bolus dose was based on the values for the apparent volume of distribution found in the literature (Nightingale *et al.*, 1975; Nauta & Mattie, 1976; Mandell & Sande, 1980). The plasma level thus obtained was maintained by a continuous i.v. infusion administered by a Razel pump (Razel Scientific Instruments; Stanford, Conn. USA); the rate of infusion was based on plasma clearance values found in the literature. To reach the higher plasma level a second bolus dose was given, followed by continuous infusion at a higher rate; this was repeated for the final period of 2.5 h in which the highest level was obtained. A solution of 4.5% glucose and 0.1% NaCl was administered through the same intravenous cannula at a constant rate of 400 to 500 ml h^{-1} by an Infusomat pump (Braun Melsungen, FRG) in order to maintain a high urinary flow, thus minimizing tubular reabsorption. Moreover the volunteers were urged to drink water or milk regularly during the entire experiment. A 5-ml blood sample was taken from a cannula in the cubital vein of the other arm at 30 min intervals. The samples were centrifuged and plasma was separated and stored at −15° C. The bladder was also emptied at 30 min intervals, i.e. immediately after each blood sample was drawn. The volume of urine was recorded; the samples were kept at −15° C. Within 7 days the concentrations of antibiotic in urine and plasma were measured. The first half hour of each 2.5 h period was regarded as a period of equilibration; the results for this period were not included in the calculations.

The procedure was identical for the three antibiotics. Nine volunteers participated in the experiments with cloxacillin, six in those with benzylpenicillin and nine in those with cephradine.

Assay

Antibiotic concentrations were measured by an agar diffusion method on phosphate peptone agar (pH 6.5) as described by Mattie *et al.* (1973), using *Bacillus subtilis* ATCC 6633 as the test organism. The level of detection in plasma was 1.25, 0.032 and 0.63 mg l^{-1} for cloxacillin, benzylpenicillin and cephradine, respectively. The coefficient of variation was between 5 and 10%. Plasma creatinine concentrations were determined with a Technicon-SMAC® autoanalyser (Technicon Instruments Corp. Terrytown, New York, U.S.). For measurement of creatinine in urine a Beckmann–Astra® autoanalyser (Beckmann, Berkeley, California, U.S.) was used. The level of detection in plasma was less than 20 μmol l^{-1} and the coefficient of variation between 5 and 10%.

Protein binding

Protein binding was estimated by equilibrium dialysis in a Dianorm® apparatus (Diachema A.G., Zürich, Switzerland). This device has two chambers, each with a volume of 1 ml, separated by a membrane which is permeable for particles smaller than 5000 D and measures 2 cm^2. Before the dialysis procedure the membrane was soaked in phosphate-buffered saline. One chamber was filled with 0.7 ml of the sample and the other with a solution of the antibiotic in buffered saline at a concentration equal to the expected free concentration in the sample. Dialysis was performed under slow rotation for 4 h at 37° C; concentrations in both chambers were then assessed against standards in saline and plasma, respectively. The concentration measured in saline was regarded as the free concentration. The results were plotted according to the Scatchard equation (Goldstein, 1949):

$$Cb/Cu = Cb_{max}/K - Cb/K \qquad (1)$$

In this equation Cb and Cu are the protein-bound and free concentrations of the antibiotic, respectively; K is the dissociation constant; and Cb_{max} is the maximal binding capacity. By a least squares linear regression method values for K and Cb_{max} were obtained. With these parameters free plasma concentrations were calculated from

the measured values of the total concentration (C_{tot}) as follows

$$Cu = \tfrac{1}{2}\,(C_{tot} - K - Cb_{max} + \sqrt{(C_{tot} - K - Cb_{max})^2 + 4K.C_{tot}}) \quad (2)$$

The total concentration required to reach a certain value of Cu can be calculated from the equation

$$C_{tot} = Cu\,(1 + Cb_{max}/(Cu + K)) \quad (3)$$

Pharmacokinetic model

The rate of saturable tubular excretion (R_{tub}) is presumed to be described by the following equation (Lundquist & Wolthers, 1958; Nelson, 1962):

$$R_{tub} = R_{tub,max} \cdot Cu/(Cu + EC_{50}) \quad (4)$$

in which $R_{tub,max}$ is the maximal rate of tubular excretion and EC_{50} is a constant representing the free concentration of the antibiotic at which R_{tub} is 50% of $R_{tub,max}$. The total renal clearance of the antibiotic (CLu_R) is given by the formula

$$CLu_R = R_R/Cu \quad (5)$$

in which R_R is the total rate of urinary excretion of the antibiotic. In this equation the mean of the calculated free plasma concentrations at the beginning and at the end of each sampling period was used. For all sampling periods the creatinine clearance was also calculated. Because of the risk of carry-over effects due to incomplete bladder emptying those samples that yielded a calculated creatinine clearance far outside the range of values obtained for the individual volunteer were not included in further analysis. The glomerular filtration rate (CLu_g) for the free fraction of antibiotic was assumed to be equal to the creatinine clearance. Tubular reabsorption of the antibiotic was neglected in the calculations because of the high urinary flow of more than 500 ml h^{-1}. Tubular clearance (CLu_{tub}) could then be defined as:

$$CLu_{tub} = CLu_R - CLu_g \quad (6)$$

and the tubular excretion rate as:

$$R_{tub} = CLu_{tub} \cdot Cu \quad (7)$$

The values that fit $R_{tub,max}$ and EC_{50} the best were calculated using the values obtained for R_{tub} and Cu. For this purpose two different methods were used:

1. With the help of an iterative computer program (NONLIN) the best fits for $R_{tub,max}$ and EC_{50} in equation 4 were established for each individual volunteer. In these calculations Cu was weighted with a factor $1/Cu$.
2. By linear regression analysis of a Scatchard plot, according to

$$R_{tub}/Cu = R_{tub,max}/EC_{50} - R_{tub}/EC_{50}, \quad (8)$$

the best fits for $R_{tub,max}$ and EC_{50} were established for each individual volunteer. Analysis of covariance was applied to establish the common slope for all volunteers, and, by that, a common value of the EC_{50}. Multiple regression analysis of values of R_{tub}/Cu as dependent variable and R_{tub} and the reciprocal value of urinary flow as independent variables was used in order to measure the degree to which urinary flow contributed to tubular clearance of the antibiotics.

Results

Protein binding

Cloxacillin The values calculated for the maximal binding capacity (Cb_{max}) and the dissociation constant (K) are given in Table 1. Analysis of covariance of Cb/Cu with respect to Cb (equation 3) showed no significant inter-individual variation in slope (i.e. $1/K$) ($F_{7;72} = 1.17$; $P > 0.10$), yielding a common value for K of 30.0 mg l^{-1} for all subjects. This value is significantly different from 0 ($P < 0.001$), indicating concentration-dependent protein-binding. The individual values of Cb_{max}, calculated from this common value of K, are also listed in Table 1. In this table the values for volunteer RR,

Table 1 Protein binding of cloxacillin

Volunteer	Cb_{max}* (mg l^{-1})	K* (mg l^{-1})	Cb_{max}** (mg l^{-1})
AH	166	19.2	231
LH	436	53.5	265
HS	307	27.2	302
JF	300	30.8	295
WD	456	58.8	274
AB	355	32.4	337
JE	249	22.7	295
FN	278	21.7	344
Mean	320	33.4	293
(s.d.)	(90)	(14.0)	(37)
RR	330	34.5	

* Maximal binding capacity (Cb_{max}) and dissociation constant (K) were calculated from equation 1.
** Calculated by using a common value for K (30.0 mg l^{-1}).

though not different from those obtained for the others, are given separately because this data was not included in the calculations of the mean parameters of tubular excretion (see 2.1).

Benzylpenicillin In the range of concentrations used in these experiments no concentration-dependent protein-binding could be demonstrated ($P > 0.80$). Therefore free concentrations were calculated as a constant fraction of the total concentration for each individual. The percentages of protein-binding are given in Table 2.

Cephradine Protein-binding of cephradine was not concentration-dependent over the range of concentrations used ($P > 0.30$). Individual binding percentages are given in Table 3.

Tubular excretion

Cloxacillin The concentration of cloxacillin could be kept reasonably constant at three different concentration levels, as illustrated for one subject in Figures 1 and 2. Although in some experiments a moderate increase or decrease occurred, this did not necessitate exclusion from further calculations. The mean concentrations obtained for each volunteer are given in Table 4. The maximal tubular excretion rate ($R_{tub,max}$) and EC_{50} were calculated by two different methods (see Methods). The results are given in Table 5. The sex, age, height and weight of the subjects are listed in the first five columns. The values of $R_{tub,max}$ and EC_{50}, calculated by means of the Scatchard (equation 8) method, are given in the next two columns. The results for volunteer RR are given separately, because the value calculated for the EC_{50} was significantly higher than those found for the others. Therefore these

Table 2 Protein binding of benzylpenicillin

Volunteer	*Protein binding (% bound)*
PG	49.3
DE	53.7
FB	54.4
BB	64.0
ER	42.7
HB	48.7
Mean	53.0
(s.d.)	(7.7)

Table 3 Protein binding of cephradine

Volunteer	*Protein binding (% bound)*
HD	13.6
LP	16.5
DP	11.4
WF	6.1
DF	9.7
HP	11.3
BV	8.1
WH	11.3
JS	11.3
Mean	11.0
(s.d.)	(3.0)

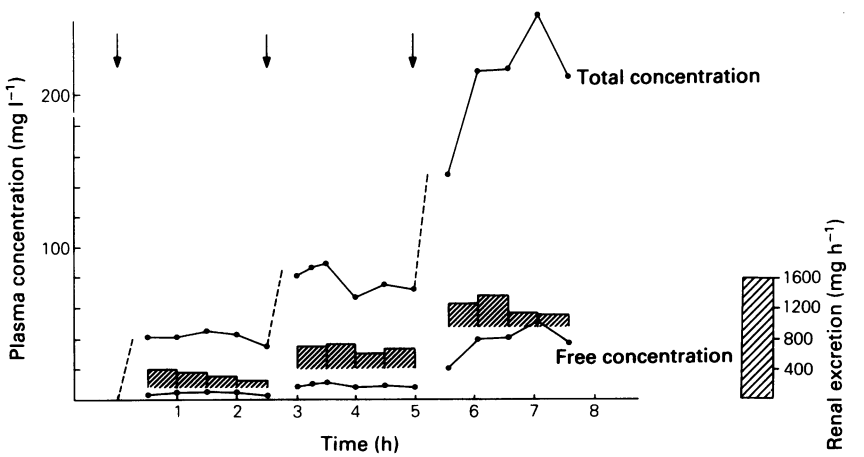

Figure 1 Renal excretion of cloxacillin in relation to the plasma concentrations during three consecutive periods of continuous infusion after a loading dose. Subject W.D. Arrows indicate the times of loading dose. Hatched columns represent the excretion rate.

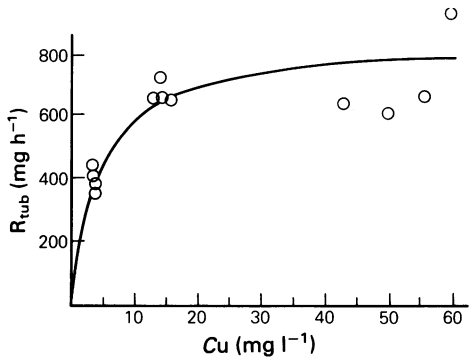

Figure 2 Calculated tubular excretion rate of cloxacillin in relation to the unbound plasma concentration. Subject W.D.

Table 4 Mean free plasma concentration of cloxacillin during three consecutive periods of continuous infusion

	Concentration (mg l^{-1})		
Volunteer	*Period 1*	*Period 2*	*Period 3*
AH	3.3	11.2	28.8
LH	0.4	2.5	29.5
HS	5.2	16.5	68.1
JF	4.5	17.7	79.5
WD	3.8	14.3	52.4
AB	3.7	12.8	78.5
JE	4.1	15.3	95.6
FN	3.4	13.7	74.4
Mean	3.6	13.0	63.4
RR	4.4	9.1	39.9

results were not included in further analysis. Analysis of covariance showed no significant variance between the slopes of the individual regression lines ($P > 0.20$). This indicates that there was no significant difference in EC_{50} between the eight remaining subjects. The common slope of the regression line differs significantly from zero ($F_{7;70} = 98.2$; $P < 0.0001$), which supports the concept of saturable tubular excretion. From the common slope a common EC_{50} of 5.9 mg l^{-1} was calculated. The values of $R_{tub,max}$ obtained by substituting the common value of EC_{50} in the Scatchard equation for each individual are shown in the last column. The correlation between Scatchard and NONLIN results for $R_{tub,max}$ was significant ($r = 0.804$; $P < 0.05$). The correlation for the individual values of EC_{50} was not significant ($r = 0.364$). The mean values according to NONLIN were 859 (s.e. mean 59) mg h^{-1} for $R_{tub,max}$ and 4.6 (s.e. mean 0.57) mg l^{-1} for EC_{50}. Although the free fraction increases when the total concentration increases, tubular clearance still decreases. For instance, at a total concentration of 100 mg l^{-1} the calculated tubular clearance would be only 107 ml min^{-1} (846 ml min^{-1} for the free fraction) Although urinary flow was in most instances sufficient to sample urine every 30 min, a substantial variation in urinary flow occurred during the experiment: the mean flow rate was about 10 ml min^{-1} but the variation coefficient within the data per volunteer was 30 to 60%. Multiple analysis of covariance of the tubular clearance with respect to the excretion rate as well as the reciprocal value of the urinary flow revealed that variance in urinary flow did not contribute significantly to the variance in tubular clearance ($P > 0.60$).

Table 5 Parameters of tubular excretion of cloxacillin: maximal tubular excretion capacity ($R_{tub,max}$) and free plasma concentration at yields 50% of $R_{tub,max}$ (EC_{50}), calculated according to equation (8)

Volunteer	*Sex*	*Age (years)*	*Height (cm)*	*Weight (kg)*	*$R_{tub,max}$ (mg h^{-1})*	*EC_{50} (mg l^{-1})*	*$R_{tub,max}$* (mg h^{-1})*
AH	M	23	180	69	891	10.50	690
LH	F	22	165	67	902	4.16	1109
HS	M	18	175	64	932	9.42	780
JF	M	21	170	63	1132	8.23	1008
WD	M	22	185	72	884	5.35	913
AB	F	20	179	58	1296	5.55	1324
JE	F	21	175	53	1151	9.72	926
FN	M	22	177	79	948	8.63	792
Mean		21	175.8	65.6	1017	7.70	943
(s.d.)		(1.4)	(6.2)	(8.1)	(155)	(2.35)	(204)
RR	M	21	193	89	1833	21.01	

* Calculated with the common value of EC_{50} (5.9 mg l^{-1}).

Benzylpenicillin In all subjects three different concentration levels were established (Table 6). The values of $R_{tub,max}$ and EC_{50} are given in Table 7. Analysis of covariance of the clearance with respect to tubular excretion showed that the slopes of the individual regression lines on the Scatchard plot did not differ significantly from one another ($F_{5,55} = 1.02$, $P > 0.05$). The common slope was significantly different from zero ($F_{1,55} = 18.8$; $P < 0.001$), as expected in the case of saturable tubular excretion. From the common slope a value for the EC_{50} was derived of 108 mg l^{-1}. The values of $R_{tub,max}$ for each individual, computed from the common EC_{50}, are also given in Table 7. There was a significant correlation between the NONLIN and Scatchard results for $R_{tub,max}$ ($r = 0.89$; $P < 0.005$). For the calculated values of the EC_{50} the correlation coefficient was on the same level of significance. The mean values according to NONLIN were 4517 (s.e. mean 1048) mg h^{-1} for $R_{tub,max}$ and 73 (s.e. mean 19) mg l^{-1} for EC_{50}. Multiple regression analysis of the tubular clearance with respect to the rate of tubular excretion and the reciprocal value of urinary flow did not reveal a contribution of urinary flow to the net tubular clearance ($P > 0.10$).

Cephradine In all subjects three different levels of plasma concentration were established. The mean levels are given in Table 8. The values of EC_{50} and $R_{tub,max}$ are given in Table 9. The difference between the slopes of the individual Scatchard plots is significant ($F_{8;87} = 3.01$; $P < 0.05$). The common slope is significantly different from zero ($F_{1;87} = 24.5$; $P < 0.001$); from this slope an EC_{50} of 266 mg l^{-1} for the free drug (299 mg l^{-1} for the total drug) was calculated. The mean values according to NONLIN were 4786 (s.e. mean 927) mg h^{-1} for $R_{tub,max}$ and 267 (s.e. mean 68) mg l^{-1} for EC_{50}. Multiple regression analysis of the tubular clearance with respect to the rate of tubular excretion and the

Table 6 Mean free plasma concentration of benzylpenicillin during three consecutive periods of continuous infusion

	Concentration (mg l^{-1})		
Volunteer	*Period 1*	*Period 2*	*Period 3*
PG	2.8	16.5	86.6
DE	2.6	8.3	41.3
FB	2.2	13.9	67.8
BB	2.1	16.5	62.4
ER	6.7	45.4	303.1
HB	2.3	18.0	75.2
Mean	3.1	19.8	106.1

The mean concentration per period is given.

Table 8 Mean free plasma concentration of cephradine during three consecutive periods of continuous infusion

	Concentration (mg l^{-1})		
Volunteer	*Period 1*	*Period 2*	*Period 3*
HD	6.7	27.4	103
LP	5.8	22.4	99
DP	5.7	27.5	115
WF	6.2	17.3	232
DF	10.5	56.9	432
HP	5.9	14.3	287
BV	6.2	20.3	105
WH	8.1	37.7	155
JS	5.5	25.0	100
Mean	6.7	27.6	181

Table 7 Parameters of tubular excretion of benzylpenicillin: maximal tubular excretion capacity ($R_{tub,max}$) and free plasma concentration that yields 50% of $R_{tub,max}$ (EC_{50}), calculated according to equation (8)

Volunteer	*Sex*	*Age (years)*	*Height (cm)*	*Weight (kg)*	*$R_{tub,max}$ (mg h^{-1})*	*EC_{50} (mg l^{-1})*	*$R_{tub,max}$* (mg h^{-1})*
PG	M	24	181	84	8121	139	6560
DE	M	23	194	70	3710	61	5630
FB	M	27	182	72	8054	119	7230
BB	M	29	193	86	7543	131	6370
ER	M	28	186	68	3283	70	4370
HB	M	23	195	91	2499	41	5350
Mean		26	189	79	5535	93	5920
(s.d.)		(3)	(6)	(10)	(2633)	(41)	(1010)

* Calculated using a common value for EC_{50} (108 mg l^{-1}).

Table 9 Parameters of tubular excretion of cephradine: maximal tubular excretion capacity ($R_{tub,max}$) and free plasma concentration that yields 50% of $R_{tub,max}$ (EC_{50}), calculated according to equation (8)

Volunteer	*Sex*	*Age (years)*	*Height (cm)*	*Weight (kg)*	$R_{tub,max}$ *(mg h^{-1})*	EC_{50} *(mg l^{-1})*	$R_{tub,max}$* *(mg h^{-1})*
HD	F	27	168	56	2350	312	2839
LP	F	21	167	61	4700	289	4367
DP	M	23	190	78	4726	261	4807
WF	F	21	181	80	11361	594	5729
DF	F	21	169	54	1952	109	3712
HP	F	20	172	60	2925	162	4353
BV	F	20	161	57	7344	449	4595
WH	M	20	196	81	1822	80	4605
JS	M	26	190	87	3652	139	6206
Mean		22	177	68	4537	266	4579
(s.d.)		(3)	(12)	(13)	(3097)	(170)	(994)

* Calculated using a common value for EC_{50} (266 mg l^{-1}).

reciprocal value of the diuresis again showed a significant negative correlation for tubular excretion ($P < 0.01$), but no significant effect of diuresis ($P > 0.10$).

Discussion

The results of the present study demonstrate that the tubular excretion of all three β-lactam antibiotics investigated is saturable. The tubular transport mechanism can be described quantitatively as a function of the maximal tubular excretion rate and the affinity of the transport system for the antibiotic, expressed as EC_{50}.

Among the methods used to calculate these parameters, the Scatchard plot was best suited for statistical analysis, because it is linear and therefore analysis of covariance and multiple regression analysis could be performed with available computer programs. However, the disadvantage of the Scatchard plot is that not only the dependent variable but also the independent variable are liable to errors of measurement of R_{tub}. Apparently a NONLIN analysis circumvents this problem, and therefore it was performed as an independent confirmation of the Scatchard analysis. Since the results obtained with the Scatchard analysis were not essentially different from those found by nonlinear regression analysis the Scatchard transformation was used for further statistical analysis. It was shown that the regression coefficient that reflects the relationship between tubular clearance (R_{tub}/Cu) and tubular excretion rate (R_{tub}) is significantly different from zero for all three antibiotics, proving that tubular excretion is indeed saturable. It should be noted that the calculated maximum rate of transport $R_{tub,max}$ is an extrapolation and should not be regarded as a measured quantity. However, the excretion rates measured in our experiments reached 80% of the calculated maximum in the case of cloxacillin. For benzylpenicillin and cephradine the measured rate was only 40% and 30%, respectively, of $R_{tub,max}$, apparently because the EC_{50} was much higher. Nevertheless, the observed values of R_{tub} for those two antibiotics were also much higher than those found for cloxacillin. The calculated $R_{tub,max}$ for cloxacillin was only one-fifth of that determined for both penicillin and cephradine, which is a highly significant difference ($P < 0.01$). The difference between penicillin and cephradine is not significant. The remarkable differences in calculated maximal transport capacity between the antibiotics suggest that there is a rate limiting step between binding to the transport system and appearance in the tubular lumen, and that this is different for the three drugs. The affinity of the tubular excretory mechanism also proved to be significantly different for the three antibiotics, the proportions of their EC_{50} values being 1:12:35 for cloxacillin, benzylpenicillin and cephradine, respectively.

The results for cloxacillin revealed that the EC_{50} for one subject was much different from those found for the others. The question thus arises whether polymorphism for the transport mechanism might exist, but only an investigation on a much larger scale can provide a definitive answer. In the cephradine group the interindividual differences were also significant, but the variation was very large so that interpretation of the results is difficult. For benzylpenicillin no significant differences were found.

In order to calculate tubular excretion from total renal excretion, it was assumed that maximal

urine flow would reduce tubular reabsorption to a negligible quantity. The literature is not unanimous on this subject. Bryner *et al.* (1948) found that urinary flow did not influence the renal excretion of benzylpenicillin. Eagle & Newman (1947) on the other hand concluded—from a small group of patients who received penicillin F, G, K or X in low doses—that clearance was dependent on diuresis. However, their results for two patients who received penicillin G by continuous infusion do not justify this conclusion. In one case a clearance of 588 ml min^{-1} at a urinary flow of 12.5 ml min^{-1} was found, while a diuresis of 2.1 ml min^{-1} yielded a clearance of 416 ml min^{-1}. In the other patient kidney function was obviously abnormal. Rantz & Kirby (1944) could not demonstrate an influence of urinary flow on the renal clearance of penicillin, but Bronfenbrenner & Favour (1945) came to the opposite conclusion. From the results of our multiple regression analysis it may be inferred that, at the relatively high flow rates maintained, variations in urinary concentration due to variations in flow had no measurable effect on tubular clearance. Although this lack of statistical evidence in itself is no proof, it does indicate that in our experiments passive tubular reabsorption was indeed minimized. The same argument holds more or less for active reabsorption, although other authors have interpreted the results of experiments carried out under different conditions such that tubular reabsorption does occur (Arvidsson *et al.*, 1979).

Some specific remarks should be added on the results for the individual antibiotics. With respect to cloxacillin our results indicate that the effect of saturation of the tubular excretory mechanism is already measurable at plasma concentrations that are not unusual in clinical practice. For instance, the EC_{50} of 5.9 mg l^{-1} represents a concentration of free cloxacillin that corresponds to a total concentration of 54.1 mg l^{-1}, as calculated from the common value of K and the mean value of 293 mg l^{-1} for Cb_{max}. At this concentration the tubular clearance of the total concentration is 147 ml min^{-1}, that of the free fraction 1332 ml min^{-1}. Since the latter value is about twice the renal plasma flow (about 700 ml min^{-1}) it is most probable that during the clearing process cloxacillin dissociates from the protein to which it is was bound. It should be noted that this conclusion is not based merely on extrapolated values for the tubular clearance, because the measured values of the clearance exceeded in many instances 1300 ml min^{-1}. The non-proportional differences in area under the curve found by Nauta & Mattie (1976) after administration of 1 and 2 g of cloxacillin can also easily be explained by saturation.

As far as benzylpenicillin is concerned, Rammelkamp & Keefer (1943) were the first to assume tubular excretion of this drug. Rantz & Kirby (1944) and Jensen *et al.* (1945) concluded that the maximal tubular excretion rate had not yet been reached at the highest plasma concentration occurring in their experiments. This is not surprising since the highest concentration was only about 0.25 mg l^{-1}. Bryner *et al.* (1948) were the first to observe decreasing renal penicillin clearance at increasing plasma concentrations. However, the authors erroneously assumed excretion to be linearly related to plasma concentration up to a certain concentration, which they defined arbitrarily as that occurring at an infusion rate of 3 000 000 u h^{-1}. From that they inferred that maximal tubular excretion should be 1800 mg h^{-1}. Their experimental design was somewhat similar to ours. The antibiotic was administered by continuous i.v. to five patients and two healthy volunteers. We reanalysed their results using the Scatchard method. The data on two patients were not included in this analysis, because their urinary data showed irregularities that indicate an abnormal renal function. The authors did not estimate glomerular filtration rate. Therefore it was not possible to correct total renal penicillin clearance for glomerular penicillin clearance, as described for our own data. The total renal clearance decreased significantly with increasing plasma concentrations ($F_{1;20} = 4.57$; $P < 0.05$). The influence of diuresis on total renal clearance was not significant. The following relation between total renal clearance and total renal excretion rate was found:

$$R_R/C(\text{ml min}^{-1}) = 583 - 0.0032 \,.\, R_R(\text{u min}^{-1})$$

Since these parameters were not corrected for glomerular filtration, they are not directly comparable with the parameters obtained with our data. From this equation values for $R_{R,max}$ (6782 mg h^{-1}) and EC_{50} (194 mg l^{-1}) can be derived, corresponding to a maximal tubular transport of about 5500 mg h^{-1} and an EC_{50} of 93 mg l^{-1} for the non-bound fraction of the drug. This is quite compatible with our results.

Hitzenberger & Spitzy (1964) also found a decrease in renal clearance at an increasing rate of infusion. Their interpretation was that this decrease does not result from limitation of the capacity of the kidney but an increase in the volume of distribution of benzylpenicillin, calculated according to a one-compartment model. However, Simon *et al.* (1971) found that the volume of the central compartment does not change. Moreover, in the calculation by Hitzenberger & Spitzy (1964) the volume of distribution is not a factor in the clearance, and therefore

cannot be brought forward as an explanation for the non-linearity of the renal excretion. Obviously the value for the maximal tubular transport of penicillin of 1800 mg h^{-1} given by Mandell & Sande (1980) is based on the conclusion of Bryner *et al.* (1948).

The results for cephradine are of interest because references to saturable excretion of the cephalosporins are scarce. Only Barza *et al.* (1976) suggest that the difference between the value for the half-life of cefamandol found in their study and that reported by Fong *et al.* (1976) could be explained by differences in the doses used for the two studies. Although it is questionable whether pharmacokinetic data from two different investigations can be compared directly, our results support the explanation given by Barza *et al.* (1976). The tubular transport of cephalosporins is also of interest because of their renal toxicity. Tune *et al.* (1977) demonstrated in animals that the nephrotoxicity of cephaloridine in animals can be diminished by concomitant infusion of substances that also undergo tubular excretion. However, this protective action was not found in man (Apple & Neu, 1977).

In conclusion, since we found very different quantitative results for these arbitrarily chosen antibiotics it may be assumed that important variations in the quantitative parameters of tubular excretion may exist between other β-lactam antibiotics, and that this should play a role in the interpretation of the results of pharmacokinetic studies.

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