Renal Clearance of Oxacillin.* (29139)

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The evaluation of an antimicrobial agent for clinical use should include an assessment of its renal clearance. Such studies have not been available heretofore for oxacillin, a new semi-synthetic, penicillinase-resistant penicillin. The renal clearance of oxacillin at different rates of infusion was determined in normal human subjects and the data are presented here.

Methods. The subjects were 16 normal male medical students and house officers. Each was studied in the evening after eating an *ad lib* meal. The subjects were encouraged to drink one to two quarts of water during the 2 hours preceding the study period. All remained in the recumbent position except when urinating. An intravenous infusion of 5% glucose in water containing the desired amount of oxacillin was begun. Large quantities of water were than given orally throughout the study to assure large urine flows. After an initial equilibration period of 20 to 30 minutes, the subject voided and began the first urine collection period. A sample of blood was drawn concomitantly; the first urine collection was ended after approximately one hour; and a second sample of blood was obtained. The second urine collection was begun immediately thereafter. A third blood sample was drawn at the end of the second collection.

The serum was separated from the blood at room temperature and frozen, as was an aliquot of each urine specimen. The creatinine concentration of a single sample of serum and urine from one of the collection periods was determined by standard methods. Renal clearances were calculated by the usual formula from drug concentration in blood and urine, and the rate of urine formation(1).

The assay of oxacillin was carried out by the serial tube dilution technique in brain-heart infusion broth. Since a single 2-fold serial dilution system tends to underestimate the amount of antibiotic present, serial 2-fold dilution systems with a basic dilution of 1:2, 1:4, etc., and 1:3, 1:6, etc., were used for determination of oxacillin concentrations in all but the first 4 patients. All urines were appropriately diluted 1:10 or 1:100 before the final dilutions were begun. Assays were carried out with group A streptococcus No. K-57 as the indicator organism. Multiple standardizations have fixed the bacteriostatic concentration of oxacillin against this streptococcus at 0.044 μg/ml of medium. Consequently, the highest dilution failing to show growth was multiplied by 0.044 to obtain the concentration of oxacillin in any particular specimen. Serial dilutions were made with 1.0 ml pipettes which were changed between successive tubes. Aliquots were then inoculated with 0.05 ml of a 1:100 dilution of a 22- to 24-hour culture of the indicator organism. To assure that the sensitivity of the indicator organism remained constant, a control with a known concentration of oxacillin was included with each lot of specimens assayed. The last clear tube before those demonstrating obvious visible growth was chosen as the end point after 24 hours of incubation. The first tube showing growth was subcultured to exclude contamination.

To evaluate the effect of serum binding of oxacillin in this assay system, concentrations of 20, 10, 5, 2.5, and 1.25 μg/ml of oxacillin were incubated in broth with and without the addition of 50% serum for 3 hours, and then assayed with 3 separate dilution systems. The results demonstrated that the oxacillin added to serum broth was recovered in the same concentration as that added to broth. This indicates that oxacillin must have become available for measurement in this system even though bound to serum protein.

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TABLE I. Oxacillin Concentrations in Blood and Urine at Various Rates of Infusion and Renal Clearances of Oxacillin and Creatinine.

Subject	Rate of infusion, g/8 hr	Concentration of oxacillin, μg/ml					Renal clearance	
		Serum			Urine		Oxacillin per square meter†	Creatinine per square meter
		1*	2*	3*	1*	2*		
R. W.	1.0	2.8	1.4	1.4	135	90	512	88
G. H.	1.0	2.8	.7	—	43	—	166	80
R. W.	1.0	1.4	1.1	.7	21	11	160	—
J. S.	1.0	2.8	2.8	2.1	43	43	175	66
A. F.	1.0	4.2	4.2	4.2	56	113	165	46
K. T.	1.0	5.6	5.6	5.6	56	84	117	70
W. I.	1.5	5.6	5.6	5.6	194	141	177	63
R. S.	1.5	4.2	8.4	8.4	282	141	103	71
L. D.	2.0	8.4	8.4	11.3	282	106	154	60
T. C.	2.0	22.6	11.3	8.4	282	141	100	57
D. H.	2.0	33.8	16.9	16.9	564	422	109	65
L. G.	2.0	22.6	16.9	16.9	282	422	131	94
M. S.	2.0	22.6	11.3	11.3	422	141	157	75
K. W.	3.0	11.3	11.3	11.3	211	282	190	70
D. G.	3.0	8.4	8.4	8.4	141	141	174	96
R. G.	3.0	11.3	22.6	16.9	423	423	118	61
Mean							169	71

* Collection periods (see text).

† Mean of 2 collection periods.

Oxacillin was administered at the rate of 1.0, 1.5, 2.0, and 3.0 g/8 hr. The infusion was regulated by counting drops per minute and making the appropriate adjustments in flow. The infusion was relatively rapid during the early portion of the equilibration period in an effort to provide a loading dose for the first 9 cases, and was then slowed to the desired infusion rate. The oxacillin concentration of many of the first samples was found to be markedly elevated over those of the last two. Consequently, this practice was not followed in the other 7 cases where the infusion rate remained constant from the beginning of experiment. All urine specimens were tested and found to be free of glucose and protein.

Results and discussion. The clearance data are tabulated in Table I. Values for serum and urine creatinine and for rate of urine production are not included. Clearance of oxacillin is presented as the mean for the 2 periods corrected for body surface area and for creatinine for a single period. Renal clearance rates were of the same order of magnitude for all subjects except R. W. regardless of the estimated infusion rate or ultimate serum concentration attained in any single instance.

The oxacillin clearance was approximately twice the creatinine clearance. Recalculation of the data obtained by Bryner *et al* for penicillin G(2), and by Smith(1) for para-aminohippuric acid, reveals an average clearance in healthy subjects of 360 cc/min/M^2, and 378 cc/min/M^2, respectively. The renal clearance of oxacillin was thus approximately 45% that of penicillin G, para-aminohippuric acid, and the renal plasma flow(1).

The data indicate that there was a tubular component to the renal clearance of oxacillin since the average value was more than twice the creatinine clearance. The contribution of glomerular clearance was difficult to assess because substantial binding to serum proteins has been demonstrated for oxacillin(3). Oxacillin is similar to phenol red in that phenol red is also highly bound to protein and has a clearance of approximately 233 cc/min/M^2 (1). Another possible explanation for the difference between the clearances of oxacillin and penicillin G is that the alteration in molecular structure of oxacillin may have reduced the rate of secretion by the renal tubules.

The data further indicate that serum binding of oxacillin must be rapidly reversible to account for the fact that 45% of the drug was removed from the serum in a single passage through the kidney. The rapid fall in

serum concentration following a single parenteral injection of oxacillin supports this thesis since little or no drug is detectable 4 to 6 hours after injection(4).

Conclusion. The renal clearance of oxacillin has been determined in normal males and found to be approximately 45% that of penicillin G. Implications regarding protein binding of oxacillin are discussed.

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Hexosamine Analysis of Renal Papillae in Diuretic and Antidiuretic Rats.* (29140)

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Ginetzinsky(1) has suggested that the action of anti-diuretic hormone (ADH) is mediated by hyaluronidase which depolymerizes the mucopolysaccharides surrounding the collecting ducts in the renal papillae, thus allowing for greater reabsorption of water from the tubules into the interstitia. He reported that the urine of rats in the anti-diuretic state contained large amounts of hyaluronidase. He also reported histological differences in the rat renal papillae between the diuretic and antidiuretic states. During diuretic states cells of the collecting tubules were plump with abundant cytoplasm and the interstitia stained very metachromatically. During anti-diuretic states cells of the tubules were flattened and seemed to have lost most of their cytoplasm, and the interstitia did not stain as metachromatically as during the diuretic state. Berlyne(2) however failed to show any relationship between states of hydration and urinary hyaluronidase. Dicker and Eggleton(3) on the other hand have confirmed the relationship between states of hydration and hyaluronidase. Thorn(4) has produced an anti-diuresis in rats by injections of large doses of hyaluronidase. Ginetzinsky's morphological findings have been disputed by Breddy *et al*(5) and have been confirmed by Stolarczyk and Manitus(6). Ivanova and Vinogradov(7) have also confirmed the histological changes and have suggested that the mucopolysaccharide involved is chondroitin sulfate C. Farber *et al*(8) have isolated acid mucopolysaccharides from beef and rabbit renal papillae.

The mucopolysaccharides isolated from the renal papillae, chondroitin sulfate and hyaluronate, contain hexosamine as one of their two repeating units. Thus the hexosamine content of the renal papillae should correlate well with the amount of acid mucopolysaccharides in the renal papillae.

This study shows that the hexosamine content of the renal papillae is correlated with the histochemical findings and with the states of diuresis and anti-diuresis of the rat. During diuresis the hexosamine content increased while during anti-diuresis the hexosamine content decreased.

Materials and methods. Over 100 white female Wistar rats, 250-300 g, fed a normal diet were divided into the following 7 groups:

Group 1 (N): normally hydrated rats, water *ad lib.*

Group 2 (D): normally hydrated rats (N) dehydrated for 24-48 hours.

Group 3 (NH): normally hydrated rats (N) further hydrated by water, (5% of body

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