Serum Binding, Distribution and Excretion of Four Penicillin Analogues Following Intravenous Injection in Man.* (26618)

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Comparative studies of many new antibiotic analogues have placed considerable emphasis upon the relative concentrations achieved in blood and on *in vitro* determinations of minimum inhibitory concentrations against specific pathogens. Such assays have been necessary because of the many difficulties inherent in performing properly designed clinical comparisons of relative therapeutic efficacy. However, conventional cup-plate assays designed to measure total antibiotic serum concentrations are somewhat deceptive in that they provide no indication of that proportion of drug which is freely diffusable. Furthermore, it is reasonable to expect that a drug which is highly bound to plasma proteins will tend to be retained to a greater extent in the intravascular compartment than one which is highly diffusable, thereby achieving higher serum concentrations, (assuming all other factors governing distribution to be equal). In certain cases, excessive serum protein binding has rendered useless otherwise promising antibiotics(1,2); serum protein binding has been shown to be of considerable importance in the pharmacology of sulfonamides(3), the penicillins(1, 2,4,5) and tetracyclines(6,7,8).

Oral absorption studies provide only limited information concerning the effect of serum protein binding on blood concentrations, and ordinarily do not give adequate information regarding the removal of drug from the blood or its clearance by the kidneys. The present study was performed to delineate the effect of the known variations in serum protein binding(5,9-11) of 4 penicillin analogues on the concentrations achieved in the blood following intravenous injection in man and on the serum decay rates and urinary recoveries following this route of administration.

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Materials and methods. Serum protein binding determinations were performed by suspending cellophane bags† containing 5 ml of pooled human serum in 10 ml of Krebs-Ringer phosphate buffer, pH 7.4, with 5, 10 or 20 units or μg of the appropriate drug. Dialysis was carried out for 24 hours at 8°C in a slowly rotating drum. Control bags contained 5 ml of buffer suspended in 10 ml of the various antibiotic solutions in buffer. Serum and buffer were removed at completion of incubation, stored at –20°C, and assayed as indicated below. All experiments were conducted in duplicate.

An intravenous dose of 500 mg of potassium benzyl penicillin‡ (G), phenoxymethyl penicillin‡ (V), phenoxyethyl penicillin‡ (Phenethicillin), (Ph) and sodium phenylmercaptomethyl penicillin§ (PM) was rapidly injected, in rotation, into 5 healthy young men. Doses were separated by at least one week. Venous blood samples were obtained at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 hours after the dose. Serums were separated as soon as possible and stored at –20°C until time of assay. All urine passed during 24 hours following the dose was collected in sterile containers and immediately refrigerated; aliquots from pools obtained at 0-1, 1-2, 2-3, 3-4, 4-8, and 8-24 hours were kept frozen at –20°C until time of assay. Specimens of serum and urine were assayed by the cup-plate method employing *Sarcina lutea* as the test organism and levels expressed in μg/ml based on comparison with standard drugs. These tests were done in the Research Laboratories, Wyeth Co. (to be referred to as Laboratory A) and the Eli Lilly Co. (Laboratory B) to which the specimens were

† Obtained from the Visking Co., Chicago, Ill.

‡ Kindly supplied with assay potency by Wyeth Laboratories.

§ Kindly supplied with assay potency by Eli Lilly Labs.

TABLE I. Human Serum Protein Binding of 4 Penicillin Analogues.

Analogue	Concentration/ml Pre-dialysis buffer	Post-dialysis Serum	Post-dialysis Buffer	% bound ± S.E.
Benzyl (G)	5 units	7.0	3.6	49.0
	"	6.3	3.3	48.3
	10	13.3	6.5	51.3
	"	10.9	5.7	47.9
	20	23.1	10.7	53.6
	"	29.7	17.1	42.3
Mean				48.7 ± 1.6
Phenoxymethyl (V)	5	6.9	1.5	78.2
	"	6.2	1.5	75.9
	10	15.9	4.7	71.7
	"	16.0	4.7	70.4
	20	31.3	7.6	75.7
	"	26.0	6.6	74.7
Mean				74.4 ± 1.2
Phenoxyethyl (phenethicillin) (Ph)	5	5.3	1.6	68.9
	"	5.6	1.4	75.0
	10	9.6	1.9	80.2
	"	9.8	1.9	81.1
	20	24.4	3.6	85.5
	"	26.3	4.6	82.5
Mean				78.8 ± 2.4
Phenylmercaptomethyl (PM)	5 μg	7.3	2.0	72.6
	" "	7.0	1.6	77.1
	10 "	20.1	3.2	84.1
	" "	15.1	3.0	80.1
	20 "	32.5	6.5	80.0
	" "	26.9	6.5	75.8
Mean				78.3 ± 1.6

delivered in the frozen state. All assays were performed on coded specimens. Laboratory A assayed concentrations of G, V and Ph in terms of each analogue while Laboratory B assayed concentrations of identical specimens of G and of PM in the same manner. Codes were not broken until results were reported to this laboratory.

Analyses of protein binding, half-life, relative distribution volumes, and tests of significance were conducted as previously reported (7).

Results. Serum binding. Serum protein binding data are summarized in Table I. Control tubes containing buffer in both bath and bag were found to have equilibrated within 24 hours. Concentrations of antibiotic pairs were either identical or within ± 10% of each other. Penicillin G was found to be the least bound to human serum (49%, differing significantly from all of the other analogues tested by 25-30%. Differences of binding between any 2 of the other analogues were not significant ($P = > .05$).

Serum levels. The average concentrations of penicillin G, V and phenethicillin in the serum of 5 subjects following an intravenous injection of 500 mg of each are presented in Fig. 1. Serum concentrations of penicillin G were somewhat lower than the other 2, but these differences are not significant. A linear relationship between the logarithm of the serum concentration *vs* time was demonstrated for each decay curve for the period of 1.5 through 4 hours following injection. Serum half-life and the y intercept at zero time were calculated by use of a regression equation(7) for each observation employing data obtained during that time period. Relative distribution volume (RDV) in liters and RDV per kg body weight in liters per kg were then calculated. These data are presented in Table II. Considerable variation was encountered among individuals and between the two different laboratories which assayed replicate samples of penicillin G in

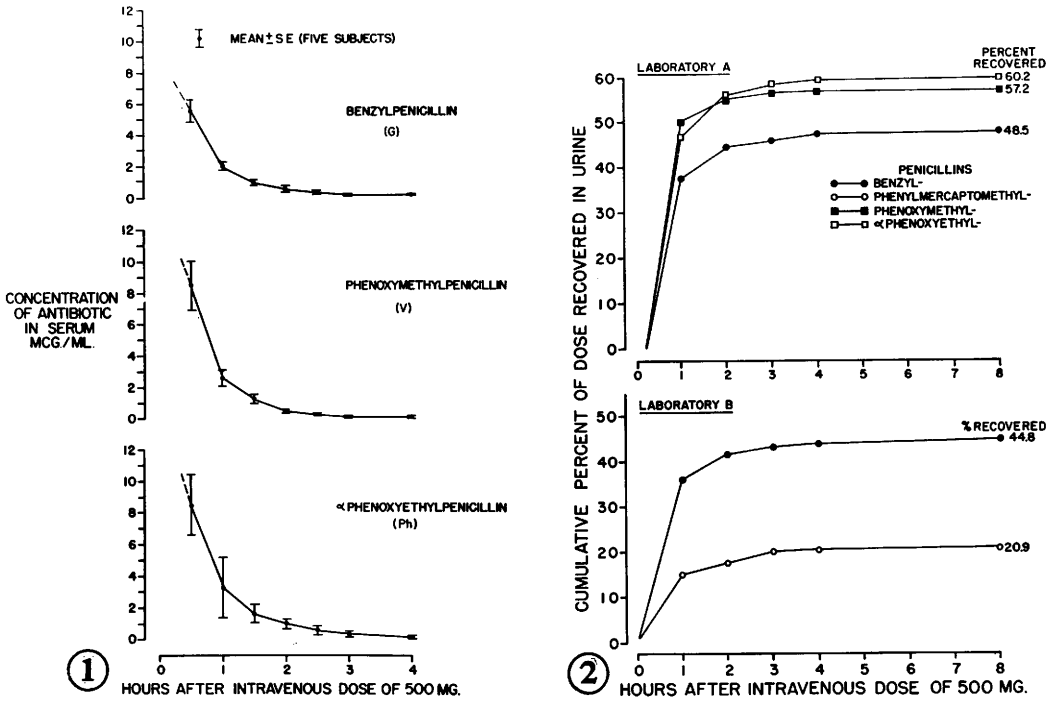

FIG. 1. Average serum concentrations of 3 penicillin analogues following intravenous injection of 500 mg of each in healthy young men (determined in Lab. A).

FIG. 2. Average cumulative 24 hr recoveries of 4 penicillin analogues following intravenous injection of 500 mg of each in healthy young men.

TABLE II. Relative Volume Distribution (RVD) of 4 Penicillin Analogues after Single Intravenous Injection.

	Lab. A				Lab. B			
Subject	Analogue	Co,† μg/ml	RVD, l	RVD/kg, l/kg	Analogue	Co, μg/ml	RVD, l	RVD/kg, l/kg
A	G*	2.6	192	2.5	G	23.3	21	.3
B		6.6	86	.9		8.1	62	.7
C		3.8	132	1.8		9.7	52	.7
D		5.8	86	1.5		4.3	116	2.0
E		1.7	294	3.8		9.6	52	.7
	Mean	4.1	156	2.1	Mean	11.0	61	.9
A	V	5.8	86	1.1	PM	6.3	79	1.0
B		4.9	102	1.3		5.8	86	1.0
C		8.3	60	.8		3.5	143	1.9
D		13.4	37	.6		9.0	56	1.0
E		7.9	63	.8		4.3	116	1.5
	Mean	8.1	70	.9	Mean	5.8	96	1.3
A	Ph	5.2	96	1.3				
B		4.7	106	1.3				
C		10.6	47	.6				
D		4.1	122	2.1				
E		5.0	100	1.3				
	Mean	5.9	94	1.3				

* The probability that the observed differences between any 2 of the above groups is due to chance is greater than .05.

† Calculated y intercept at zero time.

TABLE III. Half-Life of 4 Penicillin Analogues in Serum after Single Intravenous Injections.

Subject	Lab. A			Lab. B	
	G	V	Ph	G	PM
	Hr				
A	.68	.63	.65	.48	.57
B	.66	.53	.71	.79	.64
C	.70	.50	.66	.70	.65
D	.79	.48	1.03	1.14	.73
E	1.07	.49	.80	.64	.64
Mean	.78	.52	.77	.75	.64
± S.E.	±.15	±.03	±.13	±.11	±.02

serum. The differences encountered between any 2 groups of data were not significant.

Much more uniform results were obtained for the half-life determinations (Table III). The half-life of penicillin G calculated from data provided by assays done in both laboratories was 0.78 and 0.75 hour, respectively, and was almost identical to that of phenethicillin at 0.77 hour. The half-life of penicillin V of 0.52 hour however was significantly lower ($P = <.001$) than all the others; phenylmercaptomethyl penicillin was intermediate at 0.64 hour, a half-life significantly different from G, V and phenethicillin.

Recovery in urine. Cumulative 24 hour urinary recoveries of the 4 analogues are presented in Fig. 2. Eighty to 90% of the total amount excreted of each of the analogues was recovered in the first 1-2 hours after injection. Almost identical amounts of penicillin G (48.5 and 44.8% respectively) were recovered by each of the 2 laboratories from replicate samples. Phenethicillin was most completely recovered (60.2%), closely followed by penicillin V (57.2%). The smallest recovery was noted with mercaptomethyl penicillin, only about 21% of which was found in the urine.

Discussion. The human serum protein binding of penicillin V and G reported here is almost identical to that measured by Smith *et al.*(9); the values for penicillin G are similar to those obtained by others(5,10,12). Pindell *et al.*(11), working with relatively large amounts of these 2 analogues (mg quantities) in canine serum, found binding of each to be only about one-half that noted by others, including ourselves, in human serum. In the present study phenethicillin and phenylmercaptomethyl penicillin were bound to about the same extent as penicillin V (70-80%) while only 49% serum binding was found with penicillin G.

No significant differences in relative distribution volume per kg body weight could be demonstrated among the analogues given intravenously to human volunteers. This was in part due to the considerable individual variations encountered between subjects and laboratories performing the assays. It would be premature to conclude from these limited observations that differences in distribution volume do not exist, but it is unlikely that the differences are greater than one-fold. Cronk and Wheatly(13), similarly, were unable to demonstrate significant differences in serum concentrations of penicillins G, V and phenethicillin following intramuscular injection in man. Holland *et al.*(14), however, concluded on the basis of intramuscular injection studies in a large group of human volunteers that the distribution volume of penicillin V was considerably greater than G, because of their finding of significantly higher serum concentrations achieved with the latter drug. It is possible that the results of these authors were due to incomplete absorption of penicillin V or some other factor related to the assay of this analogue, since the recovery of penicillin V in urine was only one-half of that found in the present study while the recovery of penicillin G was in accord with our observations and those of McCarthy and Finland(15).

All 4 penicillin analogues were rapidly cleared from serum following intravenous injection, the half-life in serum of penicillin G and phenethicillin were virtually identical; phenylmercaptomethyl penicillin was removed somewhat more rapidly; penicillin V was the most rapidly cleared analogue. Holland *et al.*(14) also noted a more rapid blood clearance of V than G.

The recovery of the 4 analogues in the urine following an intravenous injection reported here parallel the data that McCarthy and Finland obtained after giving an oral dose. The recoveries of penicillin V and phenethicillin over the same time period were about twice as great following an intravenous

dose than following an oral dose, indicating incomplete oral absorption of these analogues. It is of some interest that only 21% of the phenylmercaptomethyl analogue was recovered following an intravenous injection in the present study, whereas 18% was recovered in the urine by McCarthy and Finland following an oral dose. This comparison suggests that considerably more phenylmercaptomethyl penicillin was absorbed in their studies than would appear from examination of the serum or urine concentrations alone after an oral dose. This is of particular interest in view of the marked antimicrobial activity of this analogue(15,16). Employing the same considerations urinary recovery of penicillin G following oral administration is only about one-fifth that for parenterally administered drug(15).

When the effect of serum binding is considered in relation to serum concentrations achieved by the 4 analogues, it would appear that, on a weight basis, about 25-30% more free penicillin is available with penicillin G than the other analogues. This may be of some importance in considering the relative merits of these drugs for parenteral use, but in view of their much superior intestinal absorption this advantage of penicillin G is readily overcome by the other analogues when used orally.

Many additional considerations must be taken into account in the choice of a particular analogue for a particular therapeutic task, including activity weight for weight against different organisms, cost, optimal dosage and completeness of absorption.

Summary. The *in vitro* binding to human serum proteins, and the half-life in serum, relative volume distribution and urinary recovery of 4 penicillin analogues, G, V, phenethicillin, and mercaptomethyl penicillin following single intravenous injections in normal male subjects were investigated to aid interpretation of oral absorption studies. Penicillin G was found to be significantly less bound than the others (48.7% compared to 74.4 – 78.8%). Significant differences in relative distribution volumes of the 4 analogues could not be demonstrated due in large part to considerable subject and laboratory variation. The shortest half-life in serum was obtained with penicillin V (0.52 hour), the longest with penicillin G and phenethicillin (0.75-0.78 hour), while phenylmercaptomethyl penicillin was intermediate (0.64 hour). The 24-hour urinary recoveries were penicillin G 46%, V 57%, phenethicillin 60%, and phenylmercaptomethyl penicillin 21%. It is concluded from comparison of these results with those of others that differences noted in protein binding may be outweighed by other considerations such as cost, optimum therapeutic dose, relative antimicrobial activity and extent of gastro-intestinal absorption when oral preparations are used.

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