Renal Clearance of Para-Amino Salicylic Acid in the Dog.* (21924)

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(Introduced by Eric Ogden.)
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In comparing the renal clearances of para-amino salicylic acid (PAS) and inulin in man, Horne and Wilson(1) found that the former compound had a higher clearance than the latter: The excess clearance was depressed below the level of the inulin clearance and the plasma level of PAS elevated by the administration of caronamide. On the basis of these findings, it has been postulated that there is renal tubular excretion of PAS and a number of reports(2-4) have indicated that the administration of tubular blocking agents (caronamide and Benemid) will raise the level of plasma PAS in man. However, others(5-7) have failed to show persistent differences in plasma PAS levels after oral doses when Benemid is employed as a tubular blocking agent. In the present study we have investigated the renal clearance of PAS in the dog. Our results indicate that the clearance of PAS in this species is less than the glomerular filtration rate. We have furthermore found that Benemid does not depress the renal clearance of PAS.

Methods. Female dogs anesthetized with sodium pentobarbital (30 mg/kg intravenously) were used in all experiments. In prolonged PAS infusion experiments, supplementary anesthesia was given by including the barbiturate in the infusion in quantity sufficient to supply 3 mg/kg/hour.

For the determination of bladder clearances, a priming dose of 1.4-1.6 g each of sodium PAS (Parke-Davis Co.) and creatinine in 50 ml saline was followed by an infusion through a constant infusion syringe pump of the same solution at .5 ml/min. Adequate urine flow was secured by the administration of 20 ml of 10% sodium chloride solution. After a 10-30 minute period of equilibration, the bladder was washed with three 50 ml volumes of distilled water and the washings were analyzed for both PAS and creatinine. The clearance periods were then begun. Three consecutive clearance periods of 20 minutes each were run, arterial blood samples being taken at the beginning and end of each period. Aliquots of plasma and dilute urine were precipitated with cadmium hydroxide. PAS was determined on the supernate by the method of Newhouse and Klyne(8), and creatinine by the method of Bonsnes and Taussky(9). The color measurements were carried out on a Beckman DU spectrophotometer at 545 mu for creatinine. The recovery of each substance was estimated by adding a standard amount of both to blank plasma and comparing the color developed with that of the standard alone. Recovery of added PAS varied from dog to dog ranging from 63 to 99%. Duplicate determinations on any one sample, however, checked within 5%. Variable recovery of added PAS from plasma has been previously reported(8) but remains unexplained. Plasma concentrations were corrected for the PAS and creatinine recoveries. In the calculation of the bladder clearance of PAS, the plasma concentration was corrected for PAS binding. The original plasma concentration was multiplied by the ratio between ultrafilterable and total plasma PAS as determined on a separate blood sample for each dog. Plasma ultrafiltrates were made by subjecting whole blood in ¼ inch cellophane tubing to 15 lb air pressure. The ultrafiltrate was collected in two separate portions which were analyzed separately. The PAS concentrations of the ultrafiltrate did not change with time within the limits of the analytical method. In all bladder clearance experiments, the creatinine clearance was simultaneously measured. The plasma creatinine level was corrected for the small amount of creatinine binding by the use of the ultrafiltrate/plasma creatinine factor.

The volumes of distribution of PAS and

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TABLE I. Renal Clearances and Volume of Distribution of PAS and Creatinine in the Dog (9 Animals).

Dog #	Wt, kg	Cl_{cr}, ml/kg/min.	Cl_{PAS}, ml/kg/min.	$\frac{Cl_{PAS}}{Cl_{cr}}$	V_{cr},* % B.W.	V_{PAS}, % B.W.	$\frac{V_{PAS}}{V_{cr}}$
1	9.6	4.2	3.1	.75			
2	9.5	3.9	3.4	.86			
3	11.4	3.3	2.9	.87			
4	20.0	4.8	3.7	.77			
5	18.0	4.1	3.8	.93			
6	15.4	2.1	1.6	.75	45	41	.90
7	13.0	3.7	3.5	.94	43	46	1.07
8	14.9	4.2	3.4	.82	44	44	1.00
9	15.5	5.0	2.9	.58	53	52	.99

* Volumes determined by the method of Greenberg *et al.*(10).

creatinine were estimated by the method of Greenberg *et al.*(10). From the total amount of PAS and creatinine administered in the primer and infusion, was subtracted the amount found in the bladder at the time that the first plasma sample was taken. The difference, representing the total amount of PAS and creatinine in the body was divided by the equilibrium concentration of the 2 substances in unbound form. No attempt was made to estimate the content of either substance in the renal dead space, so that these estimates of volumes of distribution were falsely high(11).

In experiments employing Benemid, the bladder clearance of PAS was determined as described above. After 3 consecutive clearance periods 0.5 g of Benemid dissolved in an equivalent amount of $NaHCO_3$(12) was injected intravenously in a 50 ml volume. The pH of the solution was 7.8. After a 10-minute period the PAS clearance was again determined in 3 consecutive periods.

Results. Binding of PAS by plasma proteins. In 12 measurements of the ratio of PAS concentration in the ultrafiltrate to that in the plasma, the free PAS was found to account for 49 to 88% of the total. The average value was 67% and the standard deviation was ± 9%. This variability in the protein binding has been noted previously in the case of other salicylates(13) and the binding of PAS in human blood is likewise variable (14).

Bladder clearance of PAS. In the first 5 bladder clearance experiments, the clearance was calculated from the plasma PAS concentration corrected by the average value for protein binding. In the last 4 experiments, the actual PAS binding by the individual dog's plasma was used in the calculation. The PAS clearance values ranged from 1.5-3.8 ml/kg/min. The creatinine clearances ranged from 2.0-5.0 ml/kg/min. The ratio of PAS to creatinine clearance ranged from .58 to .94 with an average of .81. In only 1 of 37 clearance periods did the ratio exceed 1. In this period the clearance ratio was 1.1.

Effect of Benemid on the bladder clearance of PAS. The bladder clearances of PAS in the 3 Benemid experiments were 20.8, 22.5, and 47.0 ml/min before the administration of Benemid and 30.7, 29.6 and 47.0 ml/min respectively afterwards. The creatinine clearance was unchanged in the first 2 experiments and rose slightly (from 65 to 76 ml/min) in the third.

Volumes of distribution of Creatinine and PAS. The volume of distribution calculated from the equilibrium plasma concentration and the PAS balance is, as has been noted, falsely high because of the PAS contained in the renal dead space. The average value obtained in experiments in which this method was employed was 45.5% of the body weight. In the same experiments, the volume of distribution of creatinine similarly calculated (and with almost the same error) was 46% of the body weight. We have noted elsewhere (11) that the true volume of distribution of creatinine as determined by disappearance curve analysis which eliminates the dead space error is closer to 35% of the body weight. Similar analyses of disappearance curves after single injections of PAS which

are not reported here yielded almost identical values for the volume of distribution of this substance (34% of the body weight). A similar value has been reported for the dog by Way *et al.*(14).

Discussion. The results demonstrate that in the dog the clearance of PAS is less than the glomerular filtration rate as measured by the clearance of creatinine. The fact that the clearance ratio is less than one may be interpreted to indicate that tubular reabsorption of PAS occurs in excess of tubular secretion of this molecule, if indeed the latter occurs at all. The possibility that secretion and reabsorption of PAS occur together appears unlikely because of the relatively narrow range of clearance ratios observed between PAS and creatinine. We were further unable to observe the diminution of PAS clearance with Benemid which would have been expected if this material interfered with the tubular secretion of PAS. In fact, in 2 of 3 experiments, Benemid caused an increase in the clearance of PAS.

The evidence for tubular secretion of PAS upon which the rationale of the use of tubular inhibitors is based is scanty. Only Horne and Wilson(1) appear to have compared PAS clearance and filtration rates. Others have confined their studies to the measurement of plasma levels after the oral administration of PAS with or without tubular inhibitors.

The present experiments indicate that in the dog at least, there is no evident tubular excretory activity toward PAS. If, in fact, plasma levels of PAS are elevated by such inhibitors, a finding which has been questioned repeatedly(5-7), it would appear that the site of action is not on the renal tubule.

Summary. Simultaneous measurements of the renal clearance of PAS and creatinine in the dog indicate that the former substance is not excreted by the renal tubules. Benemid does not appear to diminish the renal clearance of PAS in the dog. This raises question as to the rationale of employing Benemid as an adjunct to PAS therapy in man. The volume of distribution of PAS appears to correspond closely with that of creatinine.

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