R. W., Hague, E. E., Rucker, J. T., Am. J. Physiol., 1954, v176, 445.

5. Veith, F. J., Thrower, W. B., Surg., Gynec. Obst., 1959, v109, 687.

6. Davis, J. O., Hyatt, R. E., Howell, D. S., Circulation Res., 1955, v3, 252.

7. Starzl, T. E., Gaertner, R. A., Circulation, 1955, v12, 259.

8. Wennemark, J. R., Blake, D. F., Kezdi, P., Lev, M., Circulation Res., 1962, v11, 904.

9. Wennemark, J. R., Benvenuto, R., Lewis, F. J., J. Thor. and Card. Surg., 1960, v39, 137.

10. Wennemark, J. R., Blake, D. F., Kezdi, P., Circulation Res., 1962, v10, 280.

11. Dible, J. H., Lynch, G. A., J. Path. and Bact., 1938, v46, 271.

Received December 3, 1965. P.S.E.B.M., 1966, v122.

Effect of Hydrochlorthiazide on Renal Blood Flow and Clearance of Para-Aminohippurate and Creatinine.* (31302)

SIDNEY CASSIN AND BETTY VOGH (Introduced by M. J. Fregly)
Department of Physiology, University of Florida College of Medicine, Gainesville

The introduction of chlorthiazide and its congeners has led to considerable research on the renal handling of these compounds. All of the thiazides investigated thus far seem to be secreted by similar mechanisms and at the same tubular site as para-aminohippurate (PAH), penicillin and phenol red, *i.e.*, the proximal tubule(1-5). If two of the above organic acids are administered to dogs simultaneously, it is possible in some instances, by elevating the plasma level of one of the drugs, to depress the clearance of the other. Thus, when the plasma concentration of PAH is increased to self-depression levels, the clearance of hydrochlorthiazide (HCT) may be depressed(2). Similarly, depression of both HCT and PAH by probenecid has been demonstrated(1,2). Although suggestions have been made(1,2) that thiazides depress the clearance of PAH, we are not aware that such a depression has actually been demonstrated. Depression of PAH clearance when diuretic doses of HCT are administered would certainly invalidate the use of PAH clearance for a measure of effective renal plasma flow if the depression in clearances were due to a decrease in the extraction ratio for PAH. It is the purpose of this brief report to show the effect of 3 diuretic doses of HCT on the clearance of PAH as well as on renal blood flow as measured by an electromagnetic flowmeter.

* Supported by contract AF 41 (609)-2421.

Materials and methods. Sixteen mongrel dogs of both sexes were anesthetized with sodium pentobarbital (30 mg/kg) intravenously. One femoral artery was isolated and cannulated for blood pressure measurements; the other for blood sampling. A catheter was also placed in a femoral vein for infusions, and in each ureter for measurement of urine flow. The left renal artery was exposed by retroperitoneal approach and an electromagnetic flow probe placed around the artery. In 2 animals bifurcation of the left renal artery necessitated use of the right renal artery.

All animals were hydrated by stomach with 50 ml/kg tap water. Creatinine (50 mg/kg) and PAH (8.4-42 mg/kg) were given as priming doses intravenously one hour prior to collection of urine samples, followed by constant infusion of creatinine (3.0 mg/ml) and PAH (1.0-3.2 mg/ml) in saline at 6 ml/min. Two 20-minute urine collections were made for control clearance measurements. HCT (5, 10 or 30 mg/kg prepared in a volume of 10-20 ml of equimolar NaOH) was then infused by vein over a 10-minute period. Two additional 20-minute urine collections were made immediately after infusion of the drug. Arterial blood was withdrawn into syringes, the dead spaces of which were filled with mepesulfate (Roche) at the mid points of the urine collection periods. It was established in early experiments that the kidney on which the flowmeter probe had been placed cleared

PAH and creatinine to the same extent as the kidney which had not been disturbed. Only those clearances measured for the kidney bearing the probe are reported here. Flowmeter measurements were averaged for each 20-minute clearance period.

At the end of each experiment, the renal artery was dissected out and used for calibration of the flow probe with the dog's own blood. Flows for calibration were varied by changing either the hydrostatic pressure of a blood reservoir or a resistance distal to the probe.

Creatinine was determined in urine and in trichloroacetic acid (TCA) filtrates of plasma by the alkaline picrate method(6,7). All the creatinine clearances are 15% lower than they would have been had both standards and diluted urines been acidified with TCA to the same extent as were plasma filtrates. Since our procedure was uniform throughout all the experiments, the interpretation of the results is not affected. PAH was determined in urine and TCA filtrates of plasma by the Bratton-Marshall reaction(7).

Data for clearance of PAH, clearance of creatinine, the clearance ratio (clearance of PAH/clearance of creatinine) and for the electromagnetic blood flow were subjected to analysis of variance(8) using a randomized block design. In each series 2 periods before treatment and 2 periods after treatment were considered.

Results. Infusion of a low but diuretic dose of HCT (5 mg/kg) does not significantly alter renal blood flow or clearances of PAH and creatinine (Table I). The variance ratios in each case are less than the critical variance ratio for $P = 0.05$ ($F^{1}_{14/.05} = 4.60$). A typical experiment illustrating the lack of effect of this dose of HCT is shown in Table II. Although a marked diuresis is noted after administration of the drug, the alterations in clearances and blood flow are within the error of the method.

Dogs given a 10 mg/kg dose of HCT between periods 2 and 3 showed a significant reduction in renal clearances of PAH and creatinine as well as in renal blood flow.

When HCT was administered to 6 dogs at a dose of 30 mg/kg, there was a marked and statistically significant reduction in renal blood flow, clearance of PAH and clearance of creatinine. A selected experiment to demonstrate the effect of this drug dose is presented in Table III.

In each of the selected experiments (Tables II and III) plasma levels of PAH were

TABLE I. Effect of Hydrochlorthiazide on Renal Clearances of PAH and Creatinine, and Renal Blood Flow.

Dose HCT (mg/kg)	Period	Clearance of PAH* (ml/min)	Variance ratio	Clearance of creatinine (ml/min)	Variance ratio	Clearance ratio	Variance ratio	Blood flow measured by electromagnetic flow meter (ml/min)	Variance ratio
5† →	1	86.5	1.80§	34.3	0.40§	2.52	3.31§	151.8	0.13§
	2	85.3		33.1		2.58		152.2	
	3	83.1		30.0		2.77		155.4	
	4	80.5		30.9		2.60		161.0	
10‡ →	1	58.5	18.97¶	21.2	11.50¶	2.76	0.59§	137.9	4.91‖
	2	57.6		21.3		2.70		131.2	
	3	45.6		17.6		2.59		122.3	
	4	44.7		18.5		2.42		116.7	
30† →	1	99.6	46.10¶	30.9	9.80¶	3.16	39.39¶	201.2	9.13¶
	2	94.0		31.7		2.97		194.6	
	3	67.1		25.7		2.61		186.6	
	4	68.8		26.7		2.58		177.8	

* Clearances of PAH were not utilized for calculation of true renal blood flow since extraction ratios were not obtained.
† Average values for 5 dogs. ‡ Average values for 6 dogs. § Less than critical value of variance ratio at $P = .05$.
‖ Greater than critical value of variance ratio at $P = .05$. ¶ Greater than critical value of variance ratio at $P = .01$.

TABLE II. Demonstration of Lack of Depression of PAH Clearance by 5 mg/kg Hydrochlorthiazide. Dog 6265. Wt 17.6 kg.

Time (min)	Urine flow (ml/min)	Clearance of creatinine (ml/min)	PAH			Blood flow measured by electromagnetic flow meter (ml/min)
			Plasma (mg %)	Clearance (ml/min)	Clearance ratio	
0-20	.70	28.3	3.7	88.0	3.1	187
20-40	.66	29.4	3.5	87.6	3.0	191
40-50	Inject 5 mg/kg hydrochlorthiazide prepared as 10 mg/ml					
50-70	1.85	29.7	3.7	92.6	3.1	196
70-90	1.75	29.6	3.7	89.0	3.0	196

TABLE III. Demonstration of Effect of 30 mg/kg Hydrochlorthiazide on Renal Blood Flow and Clearances of PAH and Creatinine. Dog 62565. Wt 18.6 kg.

Time (min)	Urine flow (ml/min)	Clearance of creatinine (ml/min)	PAH			Blood flow measured by electromagnetic flow meter (ml/min)
			Plasma (mg %)	Clearance (ml/min)	Clearance ratio	
0-20	.68	37.2	3.3	173.0	4.7	297
20-40	.80	38.6	3.3	167.4	4.2	292
40-50	Inject 30 mg/kg hydrochlorthiazide prepared as 30 mg/ml					
50-70	2.7	34.4	4.1	131.7	3.8	265
70-90	1.9	33.0	4.1	120.5	3.7	245

maintained at 3.0-4.7 mg%. However, in other animals used in this study the plasma level of PAH was varied between 3.0 and 15.0 mg%. The effect of the drug on either blood flow or clearances was not altered by changes in plasma PAH concentrations in this range.

Discussion. The studies of Beyer and Baer (1-3) demonstrate clearly that probenecid depresses the renal clearance ratios of several thiazides which presumably share a common route of excretion. Similarly(1), the depression of the clearance ratio of PAH by probenecid has been established. Since it is generally assumed(1-5) that these and other organic acids utilize the same tubular site and mechanism for secretion, Beyer and Baer(1) inferred that HCT depressed reversibly the renal tubular secretion of PAH. As a result of this speculation there is an obvious reluctance on the part of investigators to utilize the clearance of PAH as a measure of effective renal plasma flow in the presence of HCT.

Our experiments indicate that, although competition may exist at high doses, the above view is unwarranted when HCT is administered at 5 mg/kg, the lowest dose which produces a maximal chloride excretion rate in dogs(9). We were able to increase the urinary flow rate 3 to 5 times with 5 mg/kg HCT without causing significant alteration in renal blood flow or in clearance of creatinine or PAH.

When the level of HCT administered was increased to either 10 or 30 mg/kg there were significant reductions in the clearance measurements and blood flow. It is interesting to note that the frequently utilized clearance ratio(1,2) could easily lead one to believe that the effect of HCT on the tubular transport of PAH at doses of 5 mg/kg and 10 mg/kg was the same, since at the 10 mg/kg dose the clearance of PAH and the clearance of creatinine decreased to about the same extent.

Although Beyer and Baer(2) propose that HCT may depress reversibly the tubular secretion of PAH, one may argue from their data that this may not be the case. Their Table II and Fig. 2 (ref. 1), dealing with low plasma levels of drugs, show that the per cent decrease in drug/creatinine clearance ratio caused by probenecid at a plasma level of 15 mg/100 ml is greater for HCT than for PAH. If one assumes(1) that an organic acid, the transport of which is most readily blocked by probenecid, is also the one with the lowest affinity for the transport system, it follows that PAH has a greater affinity for the sys-

TABLE IV. Comparison of Effect of Hydrochlorthiazide on Renal Blood Flow and Clearance of PAH.

Dose HCT (mg/kg)	Period following hydrochlorthiazide	% Decrease from mean control	
		Clearance of PAH	Blood flow
10	1	21.1	8.0
	2	23.0	14.0
30	1	30.7	5.5
	2	29.0	10.0

tem than HCT. At a low dose of HCT and PAH a competition for tubular secretory sites of these compounds need have no significant effect. As a result, a measurable depression in the clearance of PAH would not occur.

The decreased clearance of PAH at the high doses of HCT may be explained in several ways. The drug may compete for active tubular secretory sites for PAH and thus depress secretion. On the other hand, there may be a vascular effect of the HCT to decrease flow through the kidney. Our data support the view that both of these factors are involved in the reduction of PAH secretion. Table IV shows that the decrease in clearance of PAH, accompanying administration of HCT, can be attributed only partially to a decrease in renal blood flow.

It has been suggested(10,11) that the diuretic effect of HCT increases the amount of sodium presented to the distal tubule and macula densa, and thus triggers the renin-angiotensin mechanism to cause renal vasoconstriction. If the above were true one might anticipate a decreased blood flow through the kidney with the 5 mg/kg dose. Since we did not observe a decreased blood flow with the low dose of HCT, we suggest that the decreased blood flow accompanying administration of 10 to 30 mg/kg HCT is due to some other action of the drug.

Summary. Administration of hydrochlorthiazide at a diuretic dose (5 mg/kg) did not alter either renal hemodynamics or the clearances of PAH and creatinine. When given at 10 mg/kg or at 30 mg/kg, hydrochlorthiazide depressed significantly the clearances of PAH and creatinine. Data are presented to show that the depression of PAH clearance is due to action of HCT on both the renal blood flow and on tubular secretion of PAH.

The authors wish to thank Mrs. Emma Rotger and Mr. Raymond Gilbert for skilled assistance with experiments, Dr. William Mendenhall, Mr. Robert Beaver and Mr. Herbert Davis for help with statistical evaluation of data.

We are indebted to Dr. John E. Baer, Merck Institute for Therapeutic Research, who supplied the hydrochlorthiazide for this study.

1. Beyer, K. H., Baer, J. E., in Ciba Foundation Symposium on Enzymes and Drug Action, J. L. Mongar, A. V. S. de Reuck, eds., J. & A. Churchill, Ltd., 1962, p60.

2. ———, Pharm. Rev., 1961, v13, 517.

3. Beyer, K. H., Ann. N. Y. Acad. Sci., 1958, v71, 363.

4. Vander, A. J., Malvin, R. L., Wilde, W. S., Sullivan, L. P., J. Pharmacol. Exp. Therap., 1959, v125, 19.

5. Weiner, I. M., Mudge, G. H., Am. J. Med., 1964, v36, 743.

6. Bonsnes, R. W., Taussky, H. H., J. Biol. Chem., 1945, v158, 581.

7. Smith, H. W., Principles of Renal Physiology, Oxford Univ. Press, 1956, p196.

8. Goldstein, A., Biostatistics, Macmillan Co., 1964, p63.

9. Maren, T. H., Wiley, C. E., J. Pharmacol. Exp. Therap., 1964, v143, 230.

10. Thurau, K. Proc. Soc. Exp. Biol. and Med., 1961, v106, 714.

11. ———, Am. J. Med., 1964, v36, 698.

Received January 17, 1966. P.S.E.B.M., 1966, v122.