Measurement of Renal Hemodynamics in Man by the "Slope Method" without Urinalysis. (17890)

HALL S. TACKET AND C. RILEY HOUCK

From the Divisions of Medicine and Physiology, University of Tennessee, College of Medicine, and the John Gaston Hospital, Memphis.

Since the introduction by Homer Smith of methods for measuring renal plasma flow and glomerular filtration rate, attempts have been made by several investigators to adapt his procedures to clinical use. The usual method, as developed by Smith, involves accurately timed collections of urine, the maintenance of an intravenous infusion at a constant rate, the collection of frequent blood samples, and the analyses of both plasma and urine. The technic has not been generally applicable clinically largely because of its intricacies. Landowne(1), and Alving and Miller(2) attempted to simplify the procedure for measuring inulin or mannitol clearance, but simultaneous collections of both blood and urine specimens were still necessary. A method has been described by Earle and Berliner(3) and by Berger, Farber and Earle(4) that involves collections of blood specimens only. Nevertheless, as a preliminary step, one must determine the rate of infusion of mannitol or PAH necessary to maintain a constant blood level. Beyer(5) has successfully measured renal plasma flow by determining the clearance of sodium para-aminohippurate (PAH) following oral administration of this compound, thus obviating the necessity for maintaining a constant infusion. Newman(6) suggested a principle for determining mannitol clearance without the collection of urine. A substance is administered intravenously by a single injection. The proportion of that substance disappearing from the plasma per unit of time represents the fraction excreted by the kidney per unit of time, assuming no extrarenal loss. The product of that proportion (S) and the volume of fluid in which the total substance is distributed (Vc) is equivalent to the volume of fluid that contains the amount removed by the kidney per unit of time or, by definition, the renal clearance (C). Thus, $C = VcS$. Preliminary results from our application of this principle to measure clearances of mannitol and PAH in animals(7) and in man(8) were previously reported. The present communication consists of our expanded results and conclusions from 17 experiments on 16 patients.

Method. Patients were fasted overnight and well hydrated before the experiments. An indwelling catheter was inserted into the bladder and urine and blood blanks were collected. Precisely measured amounts of PAH (usually 1,380 mg) and mannitol* (usually 12,000 mg) were administered intravenously from calibrated syringes, and 50 minutes were allowed for equilibration of these substances in their fluid compartments. The bladder was emptied, and five blood samples were taken from the antecubital vein at 10 minute intervals. Two successive 20 minute urine collection periods were run. The bladder was irrigated with 20 cc saline and flushed with air for each collection. In 5 experiments, arterial blood samples were also drawn through an indwelling needle in the brachial artery. Plasma and urine specimens were analyzed for PAH and mannitol. Before colorimetric analysis, plasma filtrates

1. Landowne, M., and Alving, A. S., *J. Lab. Clin. Med.*, 1946, v31, 453.

2. Alving, A. S., and Miller, B. F., *Arch. Int. Med.*, 1940, v66, 306.

3. Earle, D. P., and Berliner, R. W., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 262.

4. Berger, E. Y., Farber, S. J., and Earle, D. P., *J. Clin. Invest.*, 1948, v27, 710.

5. Beyer, K. H., personal communication to the authors.

6. Newman, E. V., Bordley, J., and Winternitz, J., *Bull. Johns Hopkins Hosp.*, 1944, v75, 253.

7. Houck, C. R., *Fed. Proc.*, 1949, v8, 77.

8. Tacket, H. S., *Fed. Proc.*, 1949, v8, 153.

* Gratitude is expressed to Dr. William Boger, Sharp and Dohme, Inc., for making available supplies of sodium para-aminohippurate and mannitol for this study.

TABLE I.
Comparison of Slope Clearance with Urine Clearance of PAH and Mannitol Based on Analysis of Venous Plasma.

Subject		Mannitol			PAH				
		C_u	C_s	C_s/C_u	C_u	C_{s_m}	$C_{s_{pah}}$	C_{s_m}/C_u	$C_{s_{pah}}/C_u$
1.	Eczema	96.8	98.4	1.02	431	441	682	1.02	1.58
2.	Arthritis	72.0	103.7	1.44	244	236	301	.97	1.23
3.	No disease	77.7	80.9	1.04	334	333	544	1.00	1.63
4.	Hypertension	88.3	79.0	.90	363	236	418	.65	1.15
5.	Rheum. fever	56.8	85.5	1.51	249	254	549	1.02	2.20
6.	" "	76.7	79.2	1.03	363	393	339	1.08	.94
7.	" "	118.2	153.0	1.29	475	396	854	.83	1.80
8.	Arthritis	90.5	114.7	1.27	521	368	670	.71	1.29
9.	Lymphosarcoma	97.8	159.0	1.62	351	346	497	.99	1.42
10.	Pneumonia	102.0	121.5	1.19	576	420	671	.73	1.16
11.	Rheum. fever	150.0	172.0	1.15	579	552	1080	.95	1.87
12.	Malignancy	37.0	73.2	1.98	97	98	122	1.01	1.26
	Avg			1.29				.91	1.46

Symbols: C_u = urine clearance (cc/min.). C_s = slope clearance.
C_{s_m} = Slope clearance using volume of distribution of mannitol.
$C_{s_{pah}}$ = Slope clearance using volume of distribution of PAH.

and diluted urines were hydrolyzed with 0.4 N HCl for 3½ hours at 97° C to free the PAH acetylated by the liver. The plasma concentrations of PAH and mannitol in each case fell in a straight line when log of plasma concentration was plotted against time as a straight abscissa. This line, extrapolated back to the time of injection, indicated the theoretic plasma concentration of PAH and mannitol at this time. This value, divided into the total amount injected, gave the volume of distribution. Thus:

$$Vc = \frac{\text{mg injected}}{\text{P (mg/cc) at zero time}}.$$

Slope (S) was determined from the graph of the falling plasma concentrations of PAH and mannitol by the formula: $S = \frac{\ln (A-B)}{t}$ in which "A" and "B" are the plasma concentrations at two different points on the falling curve, "t" is the time interval separating those points, and "ln" is the natural logarithm. Slope clearance was determined from these data by the formula previously described, C = Vcs. For comparison, determination of simultaneous clearances by the urine collection method was made by satisfying the formula $C = \frac{UV}{P}$. P was determined 2 minutes before the mid-point of each urine collection period to allow for the lag between renal excretion and appearance of urine in the bladder. Clearances based on analysis of arterial and venous plasma were determined in 5 experiments both by the slope method and by the urine collection method. In 4 experiments, urine was collected for 24 hours and analyzed for mannitol and PAH to determine the recovery of these substances in the urine.

Results. Results are presented in Tables I, II, and III. The slope clearance of PAH determined by the volume of distribution of PAH was uniformly greater than simultaneously measured urine clearance. We considered, however, that there is justification for use of the volume of distribution of mannitol rather than of PAH in the calculation of slope clearance, since perhaps the volume of distribution of PAH cannot be accurately measured from the falling curve. Its rapid removal by the kidney from the plasma during the 50 minute mixing and equilibration period prevents plasma levels of PAH from reflecting correctly the level throughout its volume of distribution at any one time. The rapid fall of PAH concentration in the plasma gives a falsely high value for the volume of distribution of this substance. Therefore, using the volume of distribution of mannitol, we found close correlation between venous slope clearance and urine clearance in 8 of 17 ex-

TABLE II.
Comparison of PAH and Mannitol Slope Clearances of Arterial and Venous Plasmas and Correlation with Corresponding Urine Clearances.

	Mannitol							PAH							
	Arterial			Venous				Arterial			Venous				
	C_s	C_u	C_s/C_u	C_e	C_u	C_s/C_u	C_{u_v}/C_{u_a}	% recovery	C_s	C_u	C_s/C_u	C_e	C_u	C_e/C_u	C_{u_v}/C_{u_a}	% recovery
1.	172	154	1.12	186	135	1.38	.88	99	439	880	.50	478	622	.77	.71	86
2.	251	128	1.96	180	128	1.41	1.	90	474	908	.52	352	750	.47	.83	88
3.	169	129	1.31	169	129	1.31	1.	101	429	824	.52	429	824	.52	1.00	84
4.	189	191	0.99	132	134	0.99	.7	*	457	584	.79	318	485	.66	.83	*
5.	112	72	1.55	104	61	1.71	.85	98	308	445	.69	191	376	.51	.84	93
Avg			1.39			1.36	.88	97			.62			.59	.84	88

* Urine accidently discarded. Symbols: C_s—Slope clearance based on volume of distribution of mannitol, C_u—Urine clearance. Diagnosis: 1. Convalescent pneumonia; 2. Schizophrenia; 3. Possible tuberculosis; 4. Hyperthyroidism; 5. Plasma cell myeloma.

TABLE III.
Uncorrected Urine Clearances of PAH (in cc/min.) in 2 Successive 20 Minute Periods with Falling Plasma Concentrations.

Patient	C_1	C_2	C_2/C_1
J.	485	447	.92
L.	259	229	.88
M.L.	335	332	.99
G.	365	360	.99
M.P.	225	272	1.21
,,	360	366	1.02
W.	466	484	1.04
D.	490	552	1.13
G.N.	361	341	.94
C.	582	570	.98
J.A.	451	675	1.49
F.	622	675	1.08
M.M.	750	1054	1.41
V.	824	1069	1.30
E.	485	567	1.17
N.	376	492	1.31
Avg			1.12

periments. In these 8 experiments, the average quotient of slope clearance and urine clearance was 1.005. In the total experience with 17 determinations, however, the average quotient of slope clearance of PAH, based on the volume of distribution of mannitol, and urine clearance was 0.91 with a range of 0.47 to 1.08.

The correlation between slope clearance and urine clearance of mannitol was less satisfactory. The average quotient of the mannitol slope clearance and urine clearance was 1.47 with a range of 0.90 to 1.98.

Urine clearances of mannitol and PAH of venous blood, when plasma concentrations were falling, were usually smaller than simultaneous clearances of arterial blood (Table II). The average venous clearance of PAH was 84 percent of the arterial clearance, and the average venous clearance of mannitol was 88 percent of the arterial clearance.

Percentage recovery of injected PAH and mannitol is indicated in Table II. The average percentage recovery of PAH in 4 experiments was 88%, and of mannitol, 97%.

Recently, Newman(9) has presented data indicating that urine clearance of PAH is not independent of plasma concentration

9. Newman, E., Kattus, A., Genecin, A., Genest, J., Calkins, E., and Murphy, J., *Bull. Johns Hopkins Hosp.*, 1949, v84, 135.

when the plasma concentration is falling. He found that the clearance fell in successive periods as the plasma concentration of PAH decreased. Hence, we compared the urine clearances in two successive 20-minute periods as the plasma levels fell, following the 50-minute mixing and equilibration period. Results are shown in Table III. It appears that in our series, there was no consistent variation of C_{pah} with falling plasma concentration. The average ratio of the second period to the first was 1.12, indicating slightly greater clearances when the plasma level was lower.

Comment. The validity of slope clearance depends on the demonstration that no significant extra-renal loss of PAH and mannitol occurs. That such loss does not occur has been shown by Elkinton(10) and by Clark and Barker(11). To confirm this point further, we determined the plasma levels of PAH and mannitol following injections of these substances in 2 completely anuric patients with the lower nephron syndrome. After equilibration, plasma levels remained constant, indicating that with complete suppression of renal function there is no loss of PAH and mannitol by a extra-renal mechanism. Houck(12) has shown in dogs that the extra-renal clearance of PAH is 6% of the total urine clearance, and the extra-renal clearance of mannitol is 4% of the total urine clearance. Our results fail to show that mannitol clearance can be accurately determined by the slope method without urine collections. In most of our experiments, the slope clearance of mannitol was significantly lower than the simultaneously measured urine clearance. The reason for this discrepancy is not apparent. Slope clearance of PAH, based on the volume of distribution of mannitol, was more frequently confirmed by the simultaneously measured urine clearance, as shown in cases, 1, 2, 3, 5, 6, 9, 11 and 12, Table I. Although measurement of clearance by the slope method seems theoretically sound, important factors influence its accuracy. One is the possibility that plasma levels of PAH and mannitol are lower than their concentrations throughout their volumes of distribution because of their rapid removal from the plasma by the kidney. Furthermore, PAH and mannitol are likely never completely distributed because they are being excreted as they are distributed. As a result of both of these factors, the calculated volume of distribution may be too high, raising, in turn, the derived clearance value. Variation in the time between excretion of urine by the kidney and its appearance in the bladder may occur, perhaps depending upon the rate of urine flow. We arbitrarily determined the concentration of PAH 2 minutes before the midpoint of each urine collection period in calculating urine clearance. Variation in the lag more or less than 2 minutes would induce some error into the calculated clearance. Nevertheless, the close correlation between slope clearance and urine clearance observed in 8 experiments seems inexplicable by coincidence alone. The results in these instances lend encouragement to seek further the factors responsible for the lack of correlation in the remaining cases.

Our results are in accord with the experience of Brun(13) and Newman(9) that venous blood clearances of diodrast, PAH, inulin, and mannitol are lower than arterial clearances when the plasma levels of these substances are falling.

Summary. 1. We have determined PAH and mannitol clearances by the slope method without urine collection in 17 experiments, on 16 patients, comparing the results with urine clearances simultaneously measured.

2. Values of PAH clearance determined by the slope method and based on the volume of distribution either of PAH or mannitol, failed to be confirmed consistently by simultaneously measured urine clearances. PAH slope clearance agreed more often with urine clearance when the slope clearance was based on the volume of distribution of mannitol than when based on the volume of distribution of PAH. This agreement occurred too frequently to be fortuitous.

10. Elkinton, J. R., *J. Clin. Invest.*, 1947, v26, 1088.

11. Clark, J. K., and Barker, H. G., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 152.

12. Houck, C. R., *Fed. Proc.*, 1949, v8, 78.

13. Brun, C., Hilden, T., and Raaschon, F., *J. Clin. Invest.*, 1949, v28, 144.

3. Values for mannitol clearance determined by the slope method were consistently lower than simultaneously measured urine clearances; therefore, mannitol clearance cannot be determined by the slope method.

4. Clearance of venous plasma when plasma concentration is falling is lower than clearance of arterial plasma.

5. Neither mannitol nor PAH is metabolized, but they are excreted exclusively by the kidney, since a high urinary quantitative recovery of each was obtained in 24 hours, and neither disappeared from the plasma of two anuric patients.

6. Although the variation was not consistent, there was an average slightly greater urine clearance at lower plasma levels of PAH than at higher levels as the plasma concentration decreased.

Received April 27, 1950. P.S.E.B.M., 1950, v74.

Pharmacology of Isomeric Thiocyanobenzoic Acids.* (17891)

JOHN C. KRANTZ, JR., C. JELLEFF CARR, AND HAROLD H. BRYANT
From Department of Pharmacology, School of Medicine, University of Maryland, Baltimore.

In a former communication(1) the authors and Tawab studied the pharmacologic responses to meta- and para-thiocyanobenzoic acids. In the main, the actions elicited by the two compounds were identical. Upon intravenous injection in the dog and rabbit they produced marked depressor responses. These were accompanied by electrocardiographic changes. The meta- and para-thiocyanobenzoic acids relaxed smooth muscle. In low concentrations they inactivated brain dehydrogenases and cytochrome oxidase. This effect was not shared by the thiocyanate ion. Through the kindness of Dr. G. F. D'Alelio† a sample of orthothiocyanobenzoic acid was provided. It is the most difficult of the three isomers to prepare. We decided to compare its pharmacologic activity with that of the meta and para compounds. Experimental studies were conducted with the soluble sodium salts of the 3 isomeric acids.

Blood pressure studies—dogs. The blood pressure studies were conducted on 6 dogs under ether anesthesia. Carotid artery blood pressure was recorded and injections of the various compounds were made into the saphenous vein. Solutions of 0.5% and 1.0% of the sodium salts were used. In our former studies it was demonstrated that the meta- and para-thiocyanobenzoic acids produced a marked depressor response when a dose of 0.5 cc/kg of a 0.5% solution was injected. The respiration was increased in rate and amplitude. This was followed by a depression of respiration. A constant finding was a marked accentuation of the T-wave of the E.C.G. in Lead II. A typical blood pressure tracing is shown in Fig. 1. The effect of the ortho compound illustrates that this isomer does not share with the meta and para compounds the capacity of eliciting a depressor response in non-toxic doses. Larger doses produce a progressive fall in blood pressure which is invariably fatal. In contrast to the constant E.C.G. finding of T-wave accentuation with the meta and para compounds, the ortho compound produced no changes in the E.C.G. in non-toxic doses. Toxic doses caused a depression of the Q R S complex and a flattening to an inversion of the T-wave.

Action on smooth muscle. The action of the meta- and para-thiocyanobenzoic acids on the smooth muscle preparations of the rabbit's intestine and the rat's uterus *in vitro* was markedly relaxant. A concentration of

* The expense of this investigation was defrayed in part by a grant from the Bressler Alumni Research Fund.

1. Tawab, S. S. A., Carr, C. J., and Krantz, J. C., Jr., *J. Pharm. and Exp. Therap.*, 1949, v96, 416.

† Director of Research, Koppers Company, Inc., Pittsburgh, Pa.