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Correlation of Renal Clearance Techniques in Dogs: Para-Aminohippuric Acid Compared With Radio-Iodinated Hippuran[1]

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There is a growing interest in the clinical application of tests of renal function previously used in the research laboratory. Classically, determinations of renal blood flow have been based on data derived from para-aminohippuric acid (PAH) clearances. The search for a less cumbersome technique has led to the consideration of radio-iodinated hippuran (RIH) as a replacement of para-aminohippuric acid.

Comparisons of RIH clearances with PAH clearances have been reported for man under conditions of continuous, simultaneous infusion of both agents (1), as well as under conditions of single, simultaneous injections of both agents (2). Comparisons have been made of constant-infusion PAH clearances with non-simultaneous, single injection RIH clearances (3). The purpose of this communication is to report further comparisons of clearances with PAH and RIH under varying conditions in dogs.

METHODS AND MATERIALS

SIMULTANEOUS CLEARANCES: Fifteen adult mongrel dogs have been studied using renal function tests on 100 occasions over a period of 18 months. The status of the animals has changed from initial control condition, to left renal hypertrophy following right nephrectomy, to acute radiation exposure of the left kidney and then to post-radiation changes during the ensuing year.

All function studies have been accomplished with dogs under pentobarbital anesthesia administered intravenously (30 mg/Kg). Diuresis for renal clearances has been induced by the use of 4-6 per cent solutions of urea given intravenously. PAH clearances have followed the procedure outlined by Smith (4). Briefly, a loading dose of PAH is injected (6 mg/Kg.) Following this, a continuous infusion of PAH solution (100 mg%) is given to maintain a plasma concentration of approximately 2 mg per cent about 4 cc/min by drip). Twenty minutes after the administration of the priming dose and the start of the sustaining infusion, the bladder is emptied, rinsed and a 20 minute clearance period started. Blood samples are

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obtained at the start, mid-point and end of each clearance period. Simultaneously with the PAH clearance period, clearance with RIH is measured. For this, the tracer is administered as a single injection, four minutes after the loading dose of PAH, based on the schedule of 0.5 μc/Kg. For RIH, the clearance period is initiated sixteen minutes after injection of tracer.

Analyses of plasma and urine for PAH are according to the method of Smith (5). Samples of plasma and urine are analysed for I^{131} in a well-type scintillation counter under strict conditions of similar geometry.

SEQUENTIAL CLEARANCES: In addition to simultaneous clearance studies, PAH and RIH clearances have been measured on fifty-one occasions in the same dogs, at sequential times, but during the same anesthesia. Under these conditions, the procedure for PAH clearance is identical to the above. The RIH clearance is initiated approximately 30 minutes after the discontinuation of PAH sustaining solution and an hour after the PAH loading dose.

RESULTS

The data comparing 100 PAH clearances with RIH clearances when the two procedures are done simultaneously are presented in Table I. In this presentation, Status II is the situation with the animals after right nephrectomy and before irradiation of the left kidney. In general, this was a period of approximately twenty-four weeks Status III is the situation with the animals after ir-

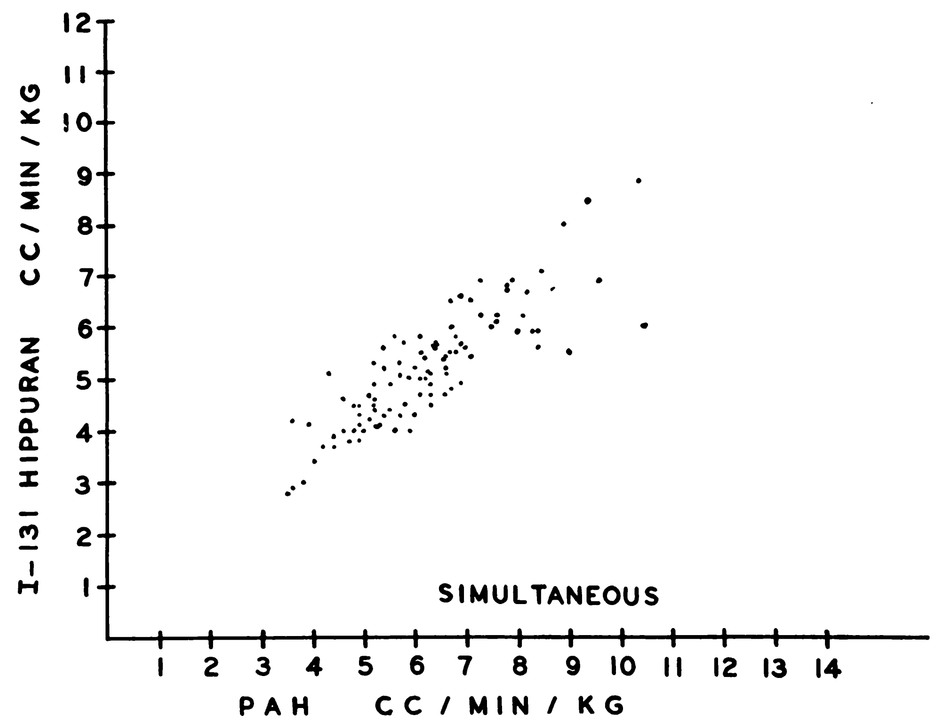

FIGURE 1

TABLE I

SIMULTANEOUS CLEARANCES PAH AND RIH

Animal	*Weight Kg*	*Status II*	*Status III*	*Urine flow cc/min*	*Clearance PAH cc/min/Kg*	*Clearance RIH cc/min/Kg*	*RIH/PAH*
#2 B	16.6	X		2.05	5.2	4.5	0.87
"	16.9	X		1.70	5.2	4.9	0.94
"	17.0	X		1.35	3.6	2.9	0.81
"	16.8	X		2.60	6.1	4.7	0.77
"	16.8	X		2.00	5.7	4.3	0.75
"	16.7	X		1.40	5.0	3.9	0.79
"	16.4		X	1.35	6.2	4.8	0.77
"	17.0		X	2.75	6.0	4.3	0.71
#3	20.4	X		1.50	4.9	4.5	0.92
"	20.4		X	0.70	5.8	5.7	0.98
"	21.0		X	2.25	6.2	5.0	0.80
"	21.0		X	2.85	6.9	4.9	0.71
"	20.5		X	1.30	6.2	4.7	0.75
"	21.5		X	0.85	3.8	4.1	1.05
#4	20.8		X	1.10	4.9	4.1	0.84
"	20.6		X	1.40	5.2	5.3	1.02
"	20.2		X	1.70	5.6	5.8	1.04
"	20.2		X	2.00	6.0	5.2	0.87
"	20.4		X	2.25	6.7	4.8	0.72
"	20.4		X	2.45	6.8	5.8	0.85
"	18.2		X	2.30	7.8	6.6	0.85
#5	17.6		X	1.60	5.1	4.2	0.82
"	19.5		X	1.50	5.2	4.4	0.85
"	20.0		X	1.60	5.5	4.4	0.80
"	19.7		X	1.42	4.9	3.8	0.76
"	19.7		X	1.35	5.9	4.0	0.69
"	19.8		X	1.00	3.4	2.8	0.81
#6	17.3		X	1.60	10.4	8.8	0.85
"	16.2		X	1.50	7.3	6.9	0.95
"	18.6		X	1.70	8.9	8.0	0.90
"	19.4		X	0.90	8.1	6.2	0.76
"	20.5		X	1.55	8.4	5.6	0.66
"	20.5		X	1.80	9.3	8.4	0.89
"	21.3		X	0.95	3.5	4.2	1.18

TABLE I—*continued*

SIMULTANEOUS CLEARANCES PAH AND RIH

Animal	*Weight* Kg	*Status* II	*Status* III	*Urine flow* cc/min	*Clearance* PAH cc/min/Kg	*Clearance* RIH cc/min/Kg	RIH / PAH
#7 B	18.3	X		1.80	6.7	6.5	0.97
"	18.9		X	1.60	7.6	6.2	0.82
"	22.4		X	0.90	4.0	3.4	0.85
"	20.8		X	1.00	4.4	3.9	0.87
"	20.8		X	2.60	6.9	5.7	0.82
"	20.5		X	1.00	5.3	4.1	0.77
"	20.8		X	1.10	5.5	4.0	0.72
#8 C	19.1	X		1.10	7.3	6.2	0.85
"	20.6	X		1.30	7.8	6.8	0.87
"	20.0	X		1.30	6.3	4.5	0.71
"	20.5		X	2.35	6.1	5.0	0.81
"	20.5		X	4.02	8.4	5.9	0.69
"	20.6		X	2.60	5.8	4.4	0.76
"	21.0		X	0.90	4.2	5.0	1.18
"	19.8		X	2.15	5.7	5.1	0.89
#9	21.8		X	1.70	7.1	6.5	0.92
"	22.0		X	1.00	6.6	5.4	0.82
#10	9.9		X	1.10	6.6	5.4	0.82
"	9.7		X	1.30	5.5	4.9	0.89
"	9.9		X	1.80	4.8	4.5	0.94
"	9.8		X	2.00	6.6	4.7	0.71
"	10.0		X	1.90	5.4	4.3	0.80
"	10.0		X	1.90	4.2	3.7	0.88
"	10.0		X	1.70	4.7	3.8	0.81
"	10.2		X	1.45	4.8	4.2	0.87
"	10.2		X	1.50	4.4	3.7	0.84
"	10.2		X	1.30	4.6	4.0	0.87
#11	20.6		X	1.20	7.9	6.9	0.87
"	19.9		X	0.90	6.1	5.5	0.90
"	19.8		X	0.80	6.4	5.7	0.89
"	22.0		X	1.60	6.4	5.7	0.89
"	22.3		X	1.70	7.0	5.6	0.80
"	22.3		X	1.20	6.8	5.5	0.81
"	23.5		X	0.70	5.2	4.6	0.88

TABLE I—*continued*

SIMULTANEOUS CLEARANCES PAH AND RIH

Animal	*Weight* Kg	*Status* II	*Status* III	*Urine flow* cc/min	*Clearance* PAH cc/min/Kg	*Clearance* RIH cc/min/Kg	RIH / PAH
#12	18.0		X	0.90	8.7	6.7	0.77
"	17.6		X	1.20	5.4	5.6	1.04
"	18.4		X	1.50	5.9	5.0	0.85
"	19.8		X	1.40	6.4	5.5	0.86
"	19.8		X	1.42	8.9	5.5	0.61
"	18.0		X	1.60	5.3	4.5	0.85
#13 B	17.1	X		1.70	8.2	6.7	0.82
"	16.8	X		2.20	7.5	6.0	0.80
"	17.3		X	3.95	9.6	6.9	0.72
"	17.3		X	2.45	6.7	5.5	0.82
"	17.4		X	1.95	6.6	6.0	0.90
"	17.4		X	1.25	8.0	5.9	0.74
"	17.9		X	2.05	7.5	6.0	0.79
"	17.4		X	2.65	6.2	5.1	0.81
#14	17.2		X	1.40	8.5	7.1	0.84
"	17.0		X	1.60	9.3	6.2	0.67
"	17.8		X	2.60	7.1	5.3	0.75
#15	12.8		X	1.20	6.9	6.6	0.96
"	12.5		X	1.60	6.1	5.8	0.95
"	13.0		X	2.30	5.7	5.3	0.93
"	13.2		X	1.50	5.4	5.2	0.96
"	13.0		X	2.00	6.3	5.1	0.81
"	13.6		X	2.20	6.6	5.2	0.79
"	13.6		X	1.70	6.6	5.1	0.77
"	13.3		X	3.95	8.3	5.9	0.71
#17 B	19.2	X		1.70	6.2	5.4	0.87
"	19.0	X		1.20	4.6	4.6	1.00
"	17.7		X	0.90	6.4	5.6	0.88
"	18.9		X	1.30	5.1	4.7	0.92
"	18.9		X	0.95	4.8	4.0	0.83
"	18.6		X	1.10	5.3	4.0	0.76
"	19.3		X	1.25	3.7	3.3	0.87

radiation of the left kidney. Clearance data are reported in cc/min/Kg as supported by Houck[6]. The range of the comparison is from 0.61 to 1.18. The mean is 0.84. Seventy-one percent of observations lie within $\pm 1\ \sigma$, ninety-three percent are within $\pm 2\ \sigma$. The ratios of RIH and PAH are presented in Figure 1.

The data comparing 51 RIH clearances with PAH clearances when the two procedures are done sequentially are presented in Table II. The range of the ratios in this series is from 0.27 to 2.56. The mean is 1.06. With this distribution of the data, thirty-three percent of the observations fall within $\pm 1\ \sigma$ and only seventy-four percent are within $\pm 3\ \sigma$. The ratios of RIH to PAH are presented in Figure 2.

The mean ratio 0.86 for simultaneous clearances of RIH to PAH is in the range of values reported by other authors under conditions of constant blood levels for both agents. A single reason for a ratio less than unity under these conditions has never been explained fully. The difference between work reported here and that of the other authors is the lack of an attempt to maintain a constant level of radio-iodinated hippuran in this work.

No attempt was made to evaluate the level of free iodine in the radio-iodinated hippuran. The material was used as received from the supplier. A report of quality control from the supplier (7) indicates levels of free iodine greater

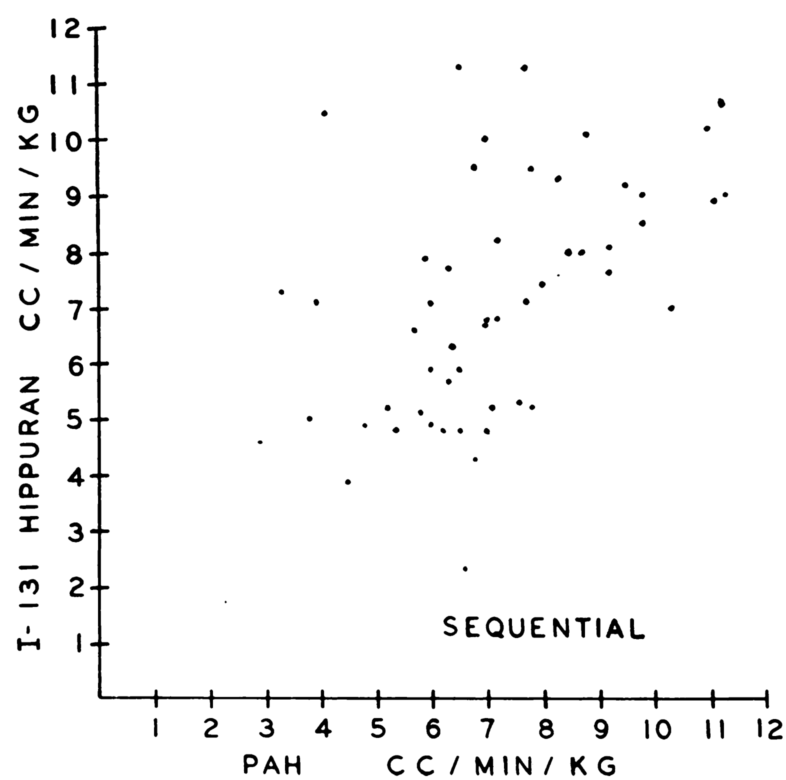

FIGURE 2

TABLE II

SEQUENTIAL CLEARANCES PAH AND RIH

Animal	*Weight Kg*	*Status II*	*Status III*	*Urine Flow PAH cc/min*	*Urine Flow RIH cc/min*	*Clearance PAH cc/min/Kg*	*Clearance RIH cc/min/Kg*	*RIH / PAH*
#3	19.1	X		2.5	2.4	6.3	7.7	1.22
"	19.2	X		1.2	1.0	2.9	4.6	1.59
"	19.2	X		0.7	1.4	3.8	5.0	1.32
"	19.9		X	1.5	1.5	6.2	4.8	0.77
"	19.6		X	0.9	1.1	6.4	6.3	0.98
#4	17.9	X		0.8	1.2	4.8	4.9	1.02
"	18.5	X		1.2	0.9	4.5	3.9	0.87
"	18.4	X		1.7	1.3	7.0	6.7	0.96
"	19.6		X	0.8	0.7	7.1	5.2	0.73
"	19.8		X	2.1	2.0	7.2	6.8	0.94
#5	16.8	X		0.6	0.9	5.4	4.8	0.89
"	18.0	X		0.5	0.9	6.8	4.3	0.63
"	19.1		X	1.5	1.7	6.0	4.9	0.82
#6	14.6	X		0.7	0.8	8.6	2.3	0.27
"	14.6	X		2.1	3.3	8.5	8.0	0.94
"	15.6	X		0.9	1.4	11.3	9.0	0.80
"	15.8		X	1.2	1.4	11.0	10.2	0.93
#7 B	16.5	X		0.9	1.3	6.3	5.7	0.91
"	17.3	X		2.0	1.7	6.5	4.8	0.74
#8 C	18.2	X		0.8	1.0	3.9	7.1	1.82
"	18.5	X		1.2	1.9	7.0	10.0	1.43
#9	19.2	X		1.3	1.4	7.8	5.2	0.67
"	19.7		X	1.8	1.9	9.8	9.0	0.92
"	20.0		X	0.8	1.2	6.0	5.9	0.98
"	20.1		X	2.0	0.7	8.0	7.4	0.93
"	21.6		X	1.2	1.3	7.7	7.1	0.92
#10	9.8	X		0.7	0.8	6.8	9.5	1.40
"	9.8	X		0.8	1.2	4.1	10.5	2.56
"	9.4	X		1.8	1.7	7.6	5.3	0.70
"	9.5	X		1.4	1.3	9.2	7.6	0.83
"	9.4		X	1.2	1.8	11.1	8.9	0.80
"	9.6		X	1.3	1.7	6.5	5.9	0.91

TABLE II—*continued*

SEQUENTIAL CLEARANCES PAH AND RIH

Animal	*Weight Kg*	*Status II*	*Status III*	*Urine Flow PAH cc/min*	*Urine Flow RIH cc/min*	*Clearance PAH cc/min/Kg*	*Clearance RIH cc/min/Kg*	*RIH / PAH*
#11	14.9	X		0.7	0.8	8.3	9.3	1.12
"	17.0	X		2.9	1.5	5.8	5.1	0.88
"	18.6		X	0.4	0.6	6.5	11.3	1.74
"	18.5		X	0.5	0.8	7.8	9.5	1.22
#12	16.4	X		2.0	2.9	5.7	6.6	1.16
"	14.8	X		2.7	2.4	7.0	4.8	0.69
"	16.7		X	1.5	1.2	9.8	8.5	0.87
"	17.8		X	1.4	2.4	6.0	7.1	1.18
#13 B	15.7	X		0.4	1.2	3.3	7.3	2.21
#14	13.2	X		1.5	1.8	7.7	11.3	1.47
"	14.6	X		1.5	1.2	9.5	9.3	0.98
"	14.6	X		1.6	1.5	9.2	8.1	0.88
"	16.0	X		0.9	1.0	10.3	7.0	0.68
"	17.4		X	1.4	1.5	8.7	8.0	0.92
#15	12.4	X		1.1	0.6	8.8	10.1	1.15
"	12.6	X		2.4	1.8	5.9	7.9	1.34
"	13.6	X		1.4	1.6	5.2	5.2	1.00
#17 B	19.0	X		1.0	1.6	7.2	8.2	1.14
"	10.5	X		1.3	1.6	7.0	6.7	0.96

than 2-3 per cent have not occurred in their material during this period of study. All tracers used in this work have been no more than three weeks old.

Problems of hippuran binding to red cells and to albumin have been considered by Burbank *et al* (1), as well as by Schwartz and Madeloff (8). It has been felt that what binding takes place will not render the substance unavailable for tubular excretion and therefore does not contribute to the ratio of less than unity.

There is a major difference in the plasma concentrations of PAH and RIH. PAH levels are maintained at 2 mgm per cent whereas maximum RIH levels are no more than 65 μgm per cent. Essig and Taggart (9) have shown inhibition of the renal transport of PAH by o-iodo hippuric acid when the latter is present in twice PAH concentration. It would seem possible, therefore, when PAH is present in concentrations 30 times that of RIH that inhibition might also be taking place.

The possibility of suppression of radio-iodinated hippuran clearance is supported by ratios of 0.5 and less when the RIH clearance is done during T_m PAH studies where the plasma PAH levels rise to the range of 36 mg per cent. Taplin et al[10] have shown a similar response in man. This is supported further by noting our average ratio of 1.06 for PAH and RIH clearances done sequentially. The dispersion of data in the sequential clearances is so great as to weaken this argument however. The blood levels of PAH at the time of the RIH clearance, when done sequentially, are not known. These levels may have been sufficiently variable so as to make spurious the points far from the mean.

As a result of these findings, it has been thought that the presence of a significant, maintained plasma level of PAH gives stability to the accuracy of radio-iodinated hippuran clearance.

Further work is planned which will allow a comparison of the techniques reported here with those reported by others in the evaluation of effective renal plasma flow and properties of tubular function.

SUMMARY

Data is presented which gives comparison of renal clearances of PAH with those of radio-iodinated hippuran. Constant plasma levels of PAH were maintained whereas levels of radio-iodinated hippuran were not constant. Using this technique and a correction factor of 0.84 $\left(\frac{\text{radio-iodinated hippuran}}{\text{para-aminohippuric acid}}\right)$, identification of RIH clearance allows prediction of PAH clearance at least as accurately as the direct measurement of PAH by routine chemical methods.

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