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Fluorometric Microanalysis of Plasma Hippuric Acid and Comparison of Hippurate with P-Aminohippurate Clearances in Dogs.* (29221)

HOWARD C. ELLIOTT AND ALFRED A. WALKER, III
Department of Chemistry, Extension Center; and Department of Medicine, Medical Center, University of Alabama, Birmingham

The relationship of the clearance of hippuric acid to p-aminohippuric acid has never been determined(1), although there are numerous studies in the literature of the clearances of the congeners of hippuric acid. It has been assumed that the clearances of p-aminohippurate and the endogenous metabolite hippuric acid are identical. Limitations of the methodology for hippuric acid have prevented an accurate, direct comparison of the relationship between these two substances. This report is concerned with the development of a micromethod for analysis of plasma hippuric acid; and comparison of the clearances of hippuric with p-aminohippuric acid in the same dog under similar experimental conditions. Knoefel and Huang(2) have reported that hippuric acid is secreted by the renal tubules in the dog, and that it exhibits quantitative competition for transport with p-aminohippurate. Sodium hippurate has been shown to depress the phenol red/creatinine clearance ratio by Smith *et al*(3). Wu and Elliott(4) have shown that in man the excretion of hippuric acid following administration of small amounts of benzoate or hippurate can be described by first order kinetics; a relationship essential for clearance data according to Bray and White(5).

Methods. The methods used for analysis of creatinine, p-aminohippurate, inulin and urine hippuric acid are the same as those previously reported(4,6). The method for hippuric acid in plasma is based on the work of Ellman *et al*(7) who described the fluorescence of hippuric acid in 70% sulfuric acid.

Plasma hippuric acid was analyzed as follows: A protein-free filtrate of plasma is made by the slow, dropwise addition with vigorous stirring (Vortex, Scientific Industries) of 1.0 ml of 20% trichloroacetic acid to a dilution of 0.5 ml of plasma in 3.5 ml of distilled water contained in a 15 × 125 mm test tube. This vigorous treatment is necessary to release the protein bound hippurate. 60-80% of the hippurate in the plasma is protein bound at the concentrations indicated in Fig. 1. The percent binding was determined by ultrafiltration using a Filterfuge Tube† with a cellophane membrane at 2400 rpm and 5°C for 4 to 6 hours.

The mixture is centrifuged, and 0.1 ml of the filtrate is added to 3.0 ml of 70% sulfuric acid in a quartz cuvet.‡ A blank is prepared containing an equivalent amount of trichloroacetic acid in 70% sulfuric acid. The solutions are mixed slowly but thorough-

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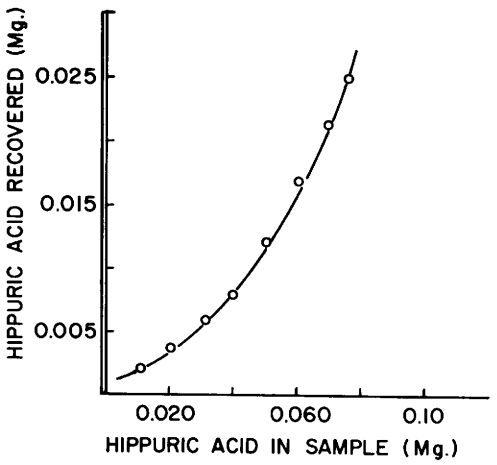
† International Equipment Co., Needham Heights, Mass.

‡ Corning Vycor #7910, 13 x 75 mm.



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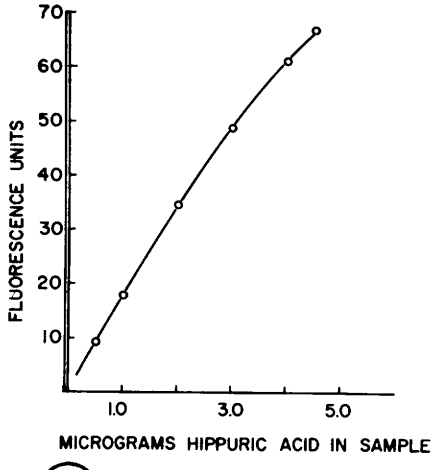

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FIG. 1. Binding of hippuric acid in normal human plasma at low concentrations using the ultrafiltration technique.
FIG. 2. Hippuric acid calibration curve.

ly by inversion of the cuvets. Fluorescence is measured against the blank in a Turner model 110 Fluorometer. A Baird-Atomic interference filter is used for activation at 250 mμ and a combination of the Corning 0-52 and 7-51 filters to provide the secondary filter at 375 mμ. The fluorophor is allowed to stand for 20 minutes, then the fluorescence is measured using the smallest slit opening. These conditions permit determination of hippuric acid in the range indicated by the calibration curve in Fig. 2. The recovery of hippuric acid added to plasma was studied. These data are illustrated in Table I. Known concentrations of hippuric acid in aqueous solution were analyzed to assess the precision of the method. The data show that the average recovery ranges from 98.4-101.0% for replicate samples in the range indicated by the calibration curve in Fig. 2. Average deviation was 1.8% of average.

Clearance determinations. The technique is essentially that described by Smith *et al* (3). Male and female dogs were anesthetized by intravenous injection of pentothal and a Cournand needle placed in the femoral artery for blood samples. Both ureters were cannulated so that clearances in each kidney could be compared. A constant infusion pump was used to maintain the plasma levels of hippuric and p-aminohippuric acids within the range of 1-4 mg % and levels of creatinine at 5-8 mg %. Creatinine clearance was used as a measure of glomerular filtration rate and 5% manitol was used to induce a small diuresis. The data from a total of 234 clearance periods are summarized in Table II.

Discussion. Ellman *et al* found(7) that the benzoylated amino acids, benzoic acid and salicyluric acid, fluoresced in 70% sulfuric acid. We have noted in using the Baird-Atomic interference filter for excitation at 250 mU that salicylic and acetylsalicylic acids do not fluoresce. P-aminohippuric acid shows approximately 15%, salicyluric acid 10% and benzoic acid almost equivalent fluorescence to hippuric acid when equal concentrations are compared. It is therefore neces-

TABLE I. Recovery of Hippuric Acid Added to Plasma.

Hippuric acid			
In plasma	Added	Found	Recovery
μg	μg	μg	%
.01	1.25	1.22	97.6
.01	2.50	2.43	97.2
.01	3.34	3.33	99.7
.02	5.00	5.32	106.4
.01	2.60	2.41	92.7
.00	1.00	.96	96.0
.57	.40	.92	95.0
.57	.80	1.40	102.0

Avg % recovery = 98.3
Range 92.7-106.4%

TABLE II. Comparison of Hippuric Acid and p-Aminohippuric Acid Clearances in the Dog.

Exp		Hippurate clearance, cc/min	p-Aminohippurate clearance, cc/min	Hipp. Cl. / Creat. Cl.	PAH Cl. / Creat. Cl.
A	r	67.8	65.2	4.03	4.08
	l	66.3	59.0	4.57	5.46
B	r	37.5	49.5	3.35	3.06
	l	35.3	51.3	3.04	3.17
C	r	33.1	33.9	3.18	3.46
	l	41.2	35.0	3.85	3.50
D	r	82.1	109.3	4.11	5.44
	l	88.6	100.0	4.06	6.04
E	r	39.3	34.4	2.77	3.00
	l	35.0	34.6	2.50	3.23
F	r	43.0	40.5	3.00	3.30
	l	41.3	46.3	2.67	3.36
G	r	22.4	22.9	2.80	3.00
	l	34.6	36.1	2.58	3.06
H	r	40.6	22.0	3.87	3.70
	l	40.5	23.0	3.58	3.71
I	r	79.0	46.3	5.98	5.12
	l	54.3	47.3	4.38	4.34
J	r	59.8	49.6	3.08	3.20
	l	56.1	47.3	2.92	3.24
K	r	57.6	60.3	2.35	3.39
	l	62.7	58.1	2.59	3.82
L	r	68.7	55.8	3.97	5.17
	l	70.7	58.7	4.00	5.29
M	r	37.4	22.4	5.19	5.44
N*		162.0	165.0	5.90	5.70
O*		175.0	206.0	2.40	2.61
Mean		51.8	48.3	3.58	3.98
S.D.		17.7	21.2	.93	.96

* Bladder clearances. Omitted from statistical analysis.

sary to prevent contamination of the plasma with these substances. Temperature change is known to affect fluorescence, and we have found it necessary to make fluorescence measurements at constant temperature in this technique to assure reproducible results.

Statistical analysis of the clearance data by standard techniques shows that there is no significant difference in the values for clearance of hippuric acid *vs* p-aminohippuric acid at the 0.05 level. The results in Table II indicate the danger of using one kidney of an experimental animal as a "control" and the other as "experimental" in studies involving small numbers of animals. The data on clearance of p-aminohippuric acid by the dog are in essential agreement with those reported by Houck(8).

Summary. 1. A method for analysis of microgram quantities of hippuric acid in plasma with good precision and accuracy is presented. 2. There is no significant difference in the clearance of hippuric acid and p-aminohippuric acid by the dog.

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Effect of Vagotomy on Renal Function During Water Diuresis.* (29222)

JOSEPH H. PERLMUTT (Introduced by John H. Ferguson)
Department of Physiology, University of North Carolina School of Medicine, Chapel Hill

Henry and coworkers(1,2) demonstrated that increased stretch of the left atrium promoted diuresis which could be inhibited by blocking vagal transmission. As a possible explanation for these results, the hypothesis has been proposed that the secretion of anti-

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