component proved to be a tripeptide that did not react with dinitrofluorobenzene and accounted for approximately 0.7 mole per mole of protein. This peptide was composed of N-terminal pyroglutamic acid, proline and C-terminal i-leucine. The presence of N-terminal pyroglutamic acid was demonstrated by alkaline hydrolysis of the tripeptide (1.0 N NaOH, 22°, 3 days) to open the pyrrolidone ring followed by dinitrophenylation, acid hydrolysis and identification of the isolated DNP-compound as DNP-glutamic acid. The absence of acetyl groups was established by the technique of Ludowieg and Dorfman (11) and by hydrazinolysis. It should be added that certain proteins and peptides(4, 12,13) have already been shown to possess pyroglutamyl as N-terminus.

Summary. A method was developed for the isolation of the N-terminal peptides of glycoproteins with N-substituted N-terminal amino acid. The N-terminal tripeptide derived from α_1-acid glycoprotein, a constituent of normal human plasma, was composed of N-terminal pyroglutamic acid, proline and C-terminal isoleucine.

1. Schmid, K., Biochim. Biophys. Acta, 1954, v14, 437.
2. Bürgi, W., Schmid, K., J. Biol. Chem., 1961, v236, 1066.
3. Ikenaka, T., Gitlin, D., Schmid, K., ibid., 1965, v240, 2863.
4. Blömback, B., Doolittle, R. F., Acta Chem. Scand., 1963, v17, 1816.
5. Cohen, S., Biochem. J., 1963, v89, 334.
6. Press, E. M., Porter, R. R., Nature, 1960, v187, 59.
7. Schmid, K., J. Am. Chem. Soc., 1953, v75, 60.
8. Li, C. H., Pertsch, L., J. Biol. Chem., 1960, v235, 2638.
9. Itano, H. A., Gottlieb, H. J., Biochem. Biophys. Res. Comm., 1963, v12, 405.
10. Narita, K., Ishii, J., J. Biochem., 1962, v52, 367.
11. Ludowieg, J., Dorfman, A., Biochim. Biophys. Acta, 1960, v37, 212.
12. Gregory, H., Hardy, P. M., Jones, D. S., Kenner, G. W., Sheppard, R. C., Nature, 1964, v204, 931.
13. Erspamer, V., Anastasi, A., Experientia, 1962, v18, 58.

Received September 29, 1965. P.S.E.B.M., 1965, v120.

Renal Handling of 5-Hydroxyindoleacetic Acid in the Dog.* (30646)

M. E. KAHIL, E. L. SIMONS, J. CASHAW AND H. BROWN (Introduced by M. Lane)
Department of Medicine, Baylor University College of Medicine, and Metabolic Research Laboratory, Veterans Administration Hospital, Houston, Texas.

The present knowledge of the renal handling of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, is incomplete. Despopoulos and Weissbach(1) have shown that rabbit kidney cortex slices, incubated in a medium containing 5-HIAA, concentrate 5-HIAA by an active aerobic process. In one patient with the carcinoid syndrome, the estimated clearance of 5-HIAA varied from 343 to 372 ml/minute, values well in excess of glomerular filtration rate, suggesting that 5-HIAA might be actively secreted into the urine by the renal tubular cells.

The purpose of this communication is to report the results of studies in which the clearance of 5-HIAA was measured in the dog.

Materials and methods. Renal clearance studies were carried out on healthy mongrel dogs of both sexes using the methods outlined by Smith(2). The dogs were anesthetized with Na Pentobarbital, 30 mg/kg administered intravenously. Blood samples were drawn from an indwelling catheter in the jugular vein. Blood was collected in chilled siliconized tubes containing heparin.

5-HIAA (Sigma) and creatinine were dis-

* This investigation was supported by USPHS Grants AM-05063-09, from Nat. Inst. of Arthritis and Metab. Dis., and FR-00134-01.

solved in a minimum volume of a 7% solution of $NaHCO_3$ and diluted to the desired volume with normal saline. After administration of the appropriate priming solution, sustaining doses of 5-HIAA and creatinine were infused at a constant rate with a Bird infusion pump. Clearances were determined at plasma levels of 5-HIAA ranging from 0.03 mg% to 86 mg%. Urine collections obtained through an indwelling bladder catheter were begun 30 minutes after the beginning of the sustaining infusion. Four 10-minute collections were obtained at each study. The bladder was washed twice with 10 ml saline after each collection period. The plasma clearance of exogeneous creatinine was used as an index of glomerular filtration rate (GFR). The same dose of creatinine(2) was used for each study. Effective renal plasma flow (RPF) was determined by the method of Wagoner *et al*(3) utilizing sodium iodohippurate I_{131} (OIH). $Hippuran_{131}$ (Abbott) contained less than one per cent of free iodide as assayed by paper chromatography and subsequent radiochromatographic scanning. One gram of probenecid in a 10% solution was rapidly infused in 5 animals at the completion of the 4th urine collection period, and a series of 10-minute clearance studies was carried out for the ensuing 90 minutes.

To determine the extent of binding of 5-HIAA to plasma protein, plasma obtained from the animals infused with different concentrations of 5-HIAA was ultrafiltered at 4°C and at 37°C in an MD 35 high pressure filtration apparatus, utilizing an LSG #60 membrane under 14 atmospheres of nitrogen. Filtration was continued until 0.5 ml of filtrate was obtained.

To determine the binding of 5-HIAA to specific proteins, lyophilized plasma protein fractions, human albumin, alpha globulin, beta globulin and gamma globulin (Pentex Corp.) were reconstituted in normal saline to concentrations of 3.3, 0.78, 0.84, and 0.66 g% respectively, and buffered to pH 7.4 with phosphate buffer. Ten μl of a solution containing one mg of 5-HIAA per ml was added to 10 ml of the various protein solutions, mixed and ultrafiltered at 37°C.

The 5-HIAA content of the plasma and filtrate was determined by the method of Sjoerdsma *et al*(4), urinary 5-HIAA by the method of Udenfriend and associates(5), and plasma and urine creatinine by the Jaffe reaction(6). The amount of 5-HIAA excreted by the tubules was calculated from the equation $T_{5\text{-HIAA}} = U_{5\text{-HIAA}}V - {}^{f}P_{5\text{-HIAA}}GFR$, ^{f}(filterable fraction) being determined by ultrafiltration at each plasma level studied. $T_{5\text{-HIAA}}$ = net tubular excretion; $U_{5\text{-HIAA}}$ = urine concentration; V = urine flow; $P_{5\text{-HIAA}}$ = plasma concentration; and GFR = glomerular filtration rate.

Results. At "low" plasma levels of 5-HIAA (.038 to 2.0 mg%) the clearance of 5-HIAA averaged 143 $\pm$ 7.8 ml/min/M^2 with simultaneous creatinine and OIH clearance of 66.0 $\pm$ 3.6 and 223 $\pm$ 20 ml/min/M^2, respectively (Table I).

At "high" plasma concentration of 5-HIAA, 20 mg% and above (Table I), the 5-HIAA clearance was markedly reduced to a mean of 85 $\pm$ 5.8 ml/min/M^2 with simultaneous creatinine and OIH clearances of 70 $\pm$ 6.5 and 259 $\pm$ 30 ml/min/M^2, respectively.

In 5 experiments, administration of one gram of probenecid (Table I) resulted in a mean decrease of the 5-HIAA clearance to 39% of the initial clearance value. In 2 of 5 experiments, the 5-HIAA clearance dropped to a value below that of the simultaneous creatinine clearance.

When plasma samples obtained from dogs infused with 5-HIAA were subjected to ultrafiltration (Table II) and both the filtrate and the nonfiltered portion analyzed for their content of 5-HIAA, 13 to 20 per cent of the plasma 5-HIAA was found to be filterable. The concentration of 5-HIAA in the nonfiltered plasma fraction was similar to that of the initial plasma (93-108%). A comparable degree of binding was observed when 5-HIAA was added to dog plasma *in vitro* and ultrafiltered.

Table III illustrates the results obtained when 5-HIAA was added *in vitro* to various solutions containing only a single protein fraction—human albumin, alpha globulin, beta globulin, and gamma globulin—and subsequently subjected to ultrafiltration. Al-

TABLE I. Simultaneous 5-HIAA, Creatinine and Sodium Iodohippurate I_{131} Clearances per M^2 in the Dog.

Plasma concentration (5-HIAA mg %)	5-HIAA	Creatinine	OIH (ml/min)	5-HIAA (after probenecid)
"Low" levels				
.038	135	67	271	72
.040	162	76	197	
.050	173	80	254	
.11	120	55	154	41
.19	121	61	211	25
1.0	162	53	287	60
1.6	126	70		
2.0	143	66	184	71
Mean ± S.E.	143 ± 7.8	66 ± 3.6	223 ± 20	
"High" levels				
20	92	94	280	
21	77	61		
47	98	91	280	
51	106	66	184	
56	76	57		
78	65	60		
86	83	59	293	
Mean ± S.E.	85 ± 5.8	70 ± 6.5	259 ± 30	

though binding occurred to each of the protein fractions, only the amount bound to albumin, 78%, approximated that in whole plasma. Lesser degrees of binding in the order of 48%, 51%, and 34% were observed with the alpha, beta, and gamma globulin fractions, respectively.

The net tubular excretion of 5-HIAA determined at several plasma concentrations in each of 3 dogs revealed that a maximal rate of tubular transport was reached at plasma 5-HIAA concentrations greater than 34 mg/100 ml. Similar results were noted when high plasma concentrations of 5-HIAA were achieved in 5 other animals (Fig. 1). The calculated maximal rate of tubular transport for 5-HIAA was 42 ± 2.3 mg/min/M^2.

TABLE II. Ultrafiltration Studies at Varying Concentrations of Plasma 5-HIAA.*

Plasma 5-HIAA (mg %)	Ultrafiltrate (mg %)	Per cent filtered	Temp °C
.037	.0062	16.7	37
.047	.0062	13.2	37
.079†	.0096	12.2	37
.079†	.0073	9.2	4
1.34	.26	19.4	4
2.47	.31	12.6	4
2.5	.46	18.0	4
19.3†	3.65	18.9	4
19.3†	4.0	20.0	37
20.2	2.7	13.4	4
46.0	7.0	15.2	4

* Uncorrected for the partial specific volume of the dissolved protein.

† 5-HIAA added *in vitro* to dog plasma.

Discussion. The results of the present study demonstrate that the clearance of 5-HIAA in the dog exceeds the GFR but is less than that of OIH, the $C_{5\text{-HIAA}}/C_{creat}$ and C_{OIH}/C_{creat} ratios being 2.9 ± 0.14 and 3.4 ± 0.4, respectively.

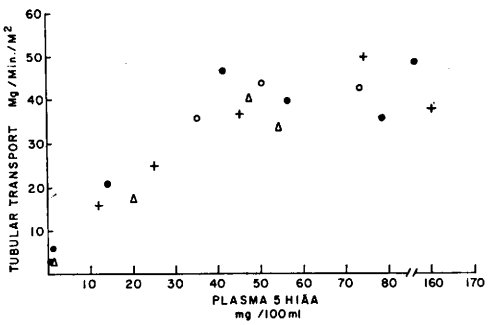

FIG. 1. Relation of plasma 5-HIAA concentration to tubular transport. ○, △, + represent serial determinations on each of 3 animals; ● individual determinations on a single dog.

Since the amount of 5-HIAA filtered by the glomerulus is only a small fraction of the 5-HIAA excreted, it is apparent that most of the urinary 5-HIAA is contributed by tubular secretion. This thesis is supported by the observed depression of 5-HIAA clearance fol-

TABLE III. *In vitro* Binding of 5-HIAA to Protein Fractions.*

Protein	5-HIAA (mg %)	Ultrafiltrate (mg %)	% filtered
Albumin	.084	.0145	17.3
(3.3 g %)	.047	.0130	27.4
Alpha globulin	.081	.037	46.3
(.84 g %)	.039	.022	57.1
Beta globulin	.074	.035	47.3
(.78 g %)	.041	.021	50.0
Gamma globulin	.068	.049	72.9
(.66 g %)	.032	.019	59.0

* Uncorrected for the partial specific volume of the dissolved protein.

lowing the administration of probenecid, a drug known to inhibit the proximal tubular secretion of many organic acids. These results are in accord with *in vitro* studies showing that rabbit renal cortical tissues concentrate 5-HIAA by an active aerobic process requiring oxidative phosphorylation, and that phenol red, which is excreted by the renal tubules, depresses the uptake of 5-HIAA by the tissues, presumably by competitive inhibition(1). Similarly, *in vivo* studies show that after subcutaneous injection of 5-HIAA, the acid is concentrated in kidney tissues. This selective concentration was inhibited by probenecid(7).

Barac(8) reported that 55% of 5-HIAA added to dog plasma was nonfilterable. In our studies, about 85% of the plasma 5-HIAA was bound to protein. In experiments utilizing various protein fractions, 5-HIAA had a somewhat greater affinity for albumin than for the other serum proteins. The ratio between the ultrafilterable and the total 5-HIAA content of plasma remained relatively constant despite marked changes in plasma 5-HIAA concentrations (0.037—46 mg%). At concentrations of 78 and 86 mg%, however, the per cent of filterable 5-HIAA increased to 30 and 44%, respectively, indicating that saturation of the plasma proteins had occurred.

Because of the role of the kidney in elimination of 5-HIAA, it can be anticipated that retention of this metabolite will occur in patients with severely impaired renal functions. Preliminary studies indicate this to be the case.

Summary. The renal clearance of 5-HIAA at low plasma concentrations in the dog averaged 143 ± 7.8 ml/min/M^2 with simultaneous creatinine and sodium iodohippurate I_{131} clearances of 66 ± 3.6 and 223 ± 20 ml/min/M^2, respectively. Probenecid administration resulted in a marked depression of the 5-HIAA clearance. Tubular secretion accounted for 88% of the 5-HIAA excreted in the urine. A tubular maximum was achieved at plasma 5-HIAA levels above 34 mg%. Ultrafiltration studies demonstrated that 80 to 87% of the plasma 5-HIAA was bound to proteins with the major portion bound to albumin.

1. Despopoulos, A., Weissbach, H., Am. J. Physiol., 1957, v189, 548.

2. Smith, H. W., in Principles of Renal Physiology, Oxford Univ. Press, New York, 1960, 196.

3. Wagoner, R., Tauxe, N., Maher, T. F., Hunt, C. J., J.A.M.A., 1964, v187, 811.

4. Sjoerdsma, A., Weissbach, H., Terry, L. L., Udenfriend, S., Am. J. Med., 1957, v23, 5.

5. Udenfriend, S., Titus, E., Weissbach, H., J. Biol. Chem., 1955, v216, 499.

6. Peters, J. H., ibid., 1942, v146, 179.

7. Milne, M. D., Crawford, M. A., Girao, C. B., Loughridge, L., Clin. Sci., 1960, v19, 165.

8. Barac, G., C. R. Soc. Biol. (Paris), 1961, v155, 1732.

Received September 9, 1965. P.S.E.B.M., 1965, v120.