sorptive mechanism would lead to the elaboration of fluid which is more representative of "precursor fluid" as in the case of the slightly hypertonic cat sweat.

Whether or not the morphologic differences in the secretory coil between man and cat have a functional counterpart is difficult to say. It is possible to speculate that because the human sweat duct extends into the secretory coil(3) and is, therefore, in close proximity with the most proximal elements of the glandular apparatus, some passage of electrolyte from duct to precursor fluid may take place. In the cat such a possibility would be precluded because the duct terminates just as the secretory coil appears in the subcutaneous tissue(9).

Summary. A method for collection of sweat under a vapor barrier from animal foot pad is presented. The average electrolyte composition of sweat from the cat foot pad was found to be (in meq/l) sodium 171, chloride 144, and potassium 23.2.

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Renal Extraction of Monosubstituted Guanidines.* (27504)

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A reduction in the maximum capacity (Tm_D) of the renal tubule to secrete iodopyracet (Diodrast®), and related substances, is the earliest and most characteristic impairment of renal function in essential hypertension. It has been suggested that Tm_D is the terminal step in a metabolic sequence, and that this impairment may represent deficiency of a phase of tubular metabolism antecedant to the process of excretion itself(1). The present studies were undertaken in an attempt to devise conditions under which one of the known synthetic functions of the renal tubule might also be evaluated as an index of early impairment in hypertension.

The renal arterial-venous concentration difference of glycocyamine was studied in man because the kidney in man appears to be the source of arterial glycocyamine(2). Since synthesis of glycocyamine utilizes arginine and in view of the fact that renal tissue from some species has the ability to synthesize arginine from citrulline(3) the renal extraction of arginine was also studied. Previous studies in man have however indicated negative renal extraction (output) of arginine as well as glycocyamine(2).

Methods. Subjects were 25 male patients with hypertensive or urologic disease (Table I) admitted to the V. A. Hospital, Richmond, Va. Twenty-two were studied in connection with the V. A. Cooperative Study of Antihypertensive Drugs(4), of whom 14 were eventually included in the study, and 10 of whom received drug therapy prior to the present studies. Patients ingested 1500 ml water during 2 hours preceding study. Right renal venous catheterization was accomplished by Seldinger's technic, with position of the catheter ascertained fluoroscopically, and verified

* Research performed during tenure of Veterans Admin. Clinical Investigatorship (1960-62).

TABLE I. Right Renal Extractions of Glycocyamine and Arginine in Individual Patients.

	Extraction,* %		Extraction,* μM/min.			C_{PAH} †	C_{IN} †
Patient	Glycocyamine	Arginine	Glycocyamine	Arginine	E_{PAH}†	E_{PAH}	C_{PAH}
G.W.	0	+20.9	− .150	+ 6.266	.45	211	.32
	+11.9	−23.4	+ .907	− 4.658			
B.W.	−15.8	− 8.2	− 1.500	− 4.458	.63	370	.18
	−14.3	− 3.7	− 1.406	− .362			
W.R.‡	+ 9.4	− 2.6	+ 1.135	− .412	.69	394	.16
	0	0	+ .120	+ 1.244			
R.G.§	+57.4	− 7.6	+32.245	− 7.757	.72	714	.15
	−35.3	+ 3.6	−13.902	+ 7.058			
M B.	− 2.3	−46.0	− .685	−22.007	.80	261	.32
	−28.4	−35.4	− 2.030	−11.404			
J.S.‖	−23.1	−27.9	− 1.443	− 5.285	.84	225	.29
	−13.7	−42.1	− .596	− 4.889			
J.A.	−21.3	−30.1	− 3.391	−11.272	.84	361	.21
	− 3.1	−20.6	+ .370	− 6.155			
A.J.	+ 2.3	−11.3	+ .319	− 7.922	.86	550	.19
	− 7.7	−15.1	− 1.512	− 7.184			
O.W.	+ 9.3	+ 2.9	+ 2.555	+21.434	.86	641	.18
	−13.8	+ 6.7	− 2.732	+ 5.018			
D.P.	−24.9	+ 3.3	− 2.237	+ 1.849	.87	262	.29
	− 5.8	−12.9	− .278	− 2.129			
W.L.¶	− 8.1	+ 3.1	− 2.119	+ 3.331	.94	758	.11
	+23.0	−11.3	+ 6.177	− 6.693			
M.P.	+25.1	+33.3	+ 3.888	+13.373	.87	361	.21
	+36.7	+42.0	+ 4.149	+14.035			
J.B.	+ 8.9	− 2.6	+ 1.498	− 1.054	.87	308	.28
	−21.9	−17.1	− 3.487	− 4.800			
L.H.	+15.4	− 8.8	+ 4.728	− 6.464	.88	636	.18
	−12.3	− 5.8	− 2.507	− 2.278			
W.E.	− 5.8	+ 7.2	− 1.084	+ 6.716	.88	473	.22
	0	+ 7.6	+ .299	+ 7.027			
A.K.**	−24.8	−19.0	− 3.482	− 7.967	.90	407	.19
	− 8.5	− 7.5	− 1.326	− 1.390			
H.L.‡	+14.7	−34.0	+ 1.387	−12.598	.90	347	.21
	−21.5	−18.4	− 1.382	− 5.047			
J.C.††	+ 8.5	− 2.6	+ 1.132	− .322	.92	541	.12
	− 5.2	− 2.9	− .403	− .049			
G.S.	−60.1	+ 1.6	−11.888	+ 1.854	.93	469	.19
	+24.1	−20.4	+ 7.277	−10.320			
E.G.‡‡	+20.3	+ 8.0	+ 5.436	+ 5.904	.94	451	.20
	+13.2	+ 2.9	+ 4.223	+ 4.485			
W.L.‡	− 7.8	− 9.1	− 1.322	− 5.126	.87	318	.12
	−46.2	−13.3	− 4.359	− 5.062			
L.B.	−21.6	−13.9	− 4.570	− 7.703	.94	534	.12
	−21.9	−24.5	− 4.845	−10.656			
W.D.	+16.1	− 4.2			.85	590	.15
	−44.8	− 2.3					
H.G.§§	+41.1	+ 1.9			.87	674	.18
	−22.2	+14.3					
W.H.	0	− 8.2			.94	531	.30
	+11.7	+12.7					

TABLE I (continued).

	Extraction,* %		Extraction,* μM/min.			C_{PAH} †	C_{IN} †
	Glycocyamine	Arginine	Glycocyamine	Arginine	E_{PAH}†	E_{PAH}	C_{PAH}
			Mean ± stand. dev.:				
Before mannitol	+ .51 ± 7.48	− 5.85 ± 5.85	+ .930 ± 7.881	− 2.667 ± 7.867	.84 ±.11	467 ±50	.20 ±.06
After mannitol	− 5.56 ± 6.29	+ 7.50 ± 7.36	− .892 ± 4.234	− 1.537 ± 6.714			
Entire group	− 3.862 ±22.59	− 6.816 ±16.97	+ .064 ± 6.282	− 2.096 ± 7.237			

* For each patient the first value shown was found before infusion of mannitol; the second immediately after infusion.

Extraction, % = $(A-R)x/Ax \times 100$.

Extraction, μM/min. = $Ax \cdot C_{PAH}/E_{PAH} - Rx \cdot C_{PAH}/E_{PAH} - UxV$.

Where E_{PAH} = Extraction ratio, $A-R_{PAH}/A_{PAH}$.

Where C_{PAH}, C_{IN}, V = Clearances of PAH, Inulin; urine flow: ml/min./1.73M².

Where Ax, Rx, Ux = Arterial, right renal venous, urinary concentration: μM/min.

† Avg of 3 successive clearance periods. (In the indicated patients, diagnosis believed to be other than primary or essential hypertension.)

‡ Chronic glomerular nephritis.

§ Left kidney atrophic, secondary to ureteral stricture; normotension.

‖ Accelerated or malignant hypertension.

¶ Acute glomerular nephritis.

** Hyperparathyroidism; hypertension.

†† Systemic lupus erythematosus; nephrotic syndrome in remission; hypertension.

‡‡ Primary myocardial disease; hypertension.

§§ Traumatic quadriplegia, 3 mo; normotension.

by sodium-p-aminohippurate (PAH) extraction. Isotonic saline solution without heparin was used to maintain patency of the catheter; 4 ml blood were withdrawn and discarded before each sampling. Initial arterial and venous samples were obtained before beginning any infusions for analysis of glycocyamine and arginine content. A second pair of samples for repeat determinations was obtained during the first renal clearance period, prior to which 100 g mannitol (25% aqueous solution) had been infused over a period of one-half hour. Additional midpoint arterial-venous paired bloods for clearance studies were obtained during 2 additional 20-minute clearance periods. Urine samples were obtained *via* multiholed urethral catheter without washouts, and aliquots saved for glycocyamine and arginine analyses. Inulin and PAH clearances are means of three 20-minute periods. Extractions of glycocyamine and arginine were calculated according to formulae given in Table I. Inulin and PAH determinations were done in duplicate using the modifications of Schreiner(5), and of Smith *et al*, (6) respectively. Glycocyamine and arginine determinations were done in duplicate by the method of Wu(7).

Results. Arterial-venous concentration differences exceeding the mean difference of replicate blood analyses (± 10%) occurred in 21 of 25 patients either before or after mannitol (Table I). When values obtaining before and after mannitol infusion are pooled, mean differences for the entire group of analyses showed negative extractions of both glycocyamine and arginine, of 3.86 and 6.82% respectively, expressed as % of arterial concentration. There was a tendency to increased negative extraction after mannitol, mean extraction of glycocyamine changing from plus 0.51 to minus 5.56%, and of arginine from minus 5.85 to minus 7.50% after mannitol. By either method of reckoning, however, the grouped values embrace wide individual variations, and the mean changes are without statistical significance. A regular increase in the small urinary excretion of arginine did occur, however, in relation to mannitol diuresis ($p<0.01$).

The figures in Table II show that all combinations of uptake and output of glycocya-

TABLE II. Breakdown of 50 Comparisons of Right Renal Veins *vs* Arterial Blood (25 Before and 25 After Mannitol Infusion Intravenously).

	1 Output glycocyamine	2 Output arginine	3 Output glycocyamine	4 Uptake arginine	5 Uptake glycocyamine	6 Output arginine	7 Uptake glycocyamine	8 Uptake arginine
No. Observations	21		8		12		9	
Mean Values	17.6%	17.3%	21.3%	7.6%	15.7%	12.5%	18.5%	12.4%

mine with uptake and output of arginine were observed. Output of glycocyamine was seen in 29 comparisons of renal venous with arterial blood, the mean value being 18.6%. In 21 of these instances, output of arginine (mean 17.3%) was also seen, and in 8 uptake (mean 7.6%) was found. In the remaining 21 observations uptake of glycocyamine (mean 16.4%) was seen; in 12, accompanying arginine output (mean 12.5%) occurred. However, in 9 instances arginine uptake rather than output 12.4% accompanied simultaneous glycocyamine uptake. With respect to arginine, in 50 observations, uptake occurred in 17 (mean 10.1%) and output in 33 (mean 15.5%).

Discussion. The object of this study was to ascertain if evaluation of the specified renal metabolic activity might prove a useful adjunct to current methods of renal assessment and/or provide additional evidence relevant to the hypothesis of Smith and coworkers. Because of the demonstrated unpredictable activity of the kidney, these results contribute nothing toward attainment of either objective. However, the results are paradoxical in the light of current concepts of renal metabolic activity and pose interesting problems of physiologic interpretation.

In studies in 6 normal, fasting non-diuresing subjects, Sandberg *et al.* found a mean negative renal extraction of glycocyamine of 22% and a mean negative extraction of arginine of 7%, and encountered no instances of uptake. These results and certain of those in the present experiments (columns 1 and 2 in Table II) are consistent with the presumed synthetic ability of the kidney for both of these compounds.

On the other hand, the results in columns 3 and 4 of Table II appear to be explainable in view of the fact that arginine is a precursor of glycocyamine. Under circumstances where the renal synthesis of arginine is less (mol for mol) than that of glycocyamine, renal uptake of arterial arginine may supply the deficit.

Turning to columns 5 and 6 of Table II, output of arginine in the presence of uptake of glycocyamine may be explained in terms of reversal of the reaction, (1) glycine + arginine $\xrightarrow{\text{transamidinase}}$ glycocyamine + ornithine(8,9,10). In the absence of renal methylferase activity such a reversal may also explain the disappearance of glycocyamine observed in columns 7 and 8. However in view of the simultaneous uptake of arginine observed also in columns 7 and 8, either reversal of the reaction, (2) arginosuccinic acid $\rightleftharpoons$ arginine + fumaric acid(11), or the presence of renal arginase activity(8) must then be postulated as routes for removal of the arginine formed as a result of reversal of reaction(1).

Another possibility, transient storage of these compounds in the kidney, may be dismissed as remote. Inclusion of these compounds in renal lymph might also have provided a positive extraction. If so, mannitol, certainly a lymphogogue since it does not enter cells, should have had a consistent effect on renal extraction, which it did not in these experiments.

A final possibility is the existence of specific renal phosphoryl transferases for phosphorylation of arginine and glycocyamine, as well as for creatine(12); considering the strong phylogenetic imprint of the kidney, and in view of the demonstrated coexistence of more than one phosphogen and of more than one phosphoryltransferase in muscle in certain invertebrate species(13). In this cir-

cumstance, uptake of arginine and glycocyamine may be regulated by cellular requirements unrelated to the known metabolic synthetic reactions; output would reflect the circumstance where synthetic capacity of the kidney is more than adequate for the demand for phosphogens. An analogous situation is seen in the renal extraction of glucose, where under some circumstances output is observed, and under other circumstances uptake of arterial glucose occurs(14).

Summary. Renal extractions of arterial glycocyamine and arginine were determined by sampling urine and right renal venous blood in 25 males studied during diuresis because of hypertensive or urologic disease. Both positive and negative extractions of both compounds were observed. The finding of negative extractions is compatible with the capacity for renal synthesis of these compounds shown *in vitro* in other species. The finding of positive extractions is discussed in terms of utilization of these compounds in local renal metabolism, in addition to previously postulated roles as substrates for extrarenal syntheses.

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Immunologic, Chemical and Isotopic Identification of C^{14}-Labeled Bacterial Polysaccharide in Animal Tissues.* (27505)

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Upon parenteral injection polysaccharides isolated from bacteria have been found to remain for many weeks in animal tissues(1-5). Slow degradation of such polysaccharides in animal tissue has been suggested by studies with isotopically labeled products(4). After the first few days following intravenous injection of C^{14}-labeled Klebsiella polysaccharide, the label was excreted slowly in the urine while the C^{14} content of tissue gradually decreased(4). Although approximately 30% of the isotopic label in animal tissue was recovered by a simple extractive procedure and found to have some of the haptenic property of the injected labeled polysaccharide(4,6), the nature of most of the label in tissue remained uncertain. The present paper concerns an improved method of extraction from animal tissue, the chemical determination of monosaccharide constituents of Klebsiella polysaccharide and identification of the recovered material through relative isotopic labeling of the 3 constituent monosaccharides as well as by haptenic properties.

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Materials and methods. Capsulated *Klebsiella pneumoniae,* type B, were harvested from the surface of agar plates and the polysaccharide extracted with 0.1N NaOH, fol-