OBSERVATIONS ON RENAL PROTEIN METABOLISM*· ‡

Renal Venous Amino Nitrogen Content before and Following Hemoglobin Infusions in Dogs

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The view that proteinuria in renal disease arises principally as a result of a reduction in the rate of renal tubular reabsorption of protein molecules, as opposed to the conventional belief in altered glomerular capillary permeability, rests upon the demonstration of a normal reabsorptive mechanism and its disturbance in disease. Histologic evidence suggests that a variety of proteins may be abstracted from glomerular filtrate in both normal and diseased kidneys (1) but there is little quantitative information concerning the rate at which this occurs. Moreover, physiologic evidence of protein reabsorption in normal circumstances is limited. For the protein hemoglobin a tubular transfer rate has been demonstrated (2), but the evidence of this for other proteins is for the most part inferential (1).

Recently Eliasch and his associates (3) have demonstrated that the amino acid and polypeptide content of renal venous blood of normal rabbits exceeded that of arterial and vena caval blood. It was suggested that this difference might arise as a result of the renal tubular reabsorption, degradation, and metabolism of filtered protein. The present investigation was undertaken in order to examine this hypothesis in dogs. The free and total amino acid content of renal venous, arterial, and inferior vena caval blood was determined in fasting animals and following an infusion of hemoglobin, a protein for which there is considerable evidence of tubular reabsorption (2, 4).

Method

Female mongrel dogs, weighing between 7 and 30 kg., were anesthetized with sodium pentobarbital, 30 mg./kg. body weight, after a 24 hour fast. An indwelling needle was inserted into a femoral artery and a multiholed venous catheter was introducted into a jugular

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‡ A preliminary report of this study has appeared previously in abstract form (14).

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vein. Under fluoroscopic control, the catheter was placed in the inferior vena cava below the renal veins, a sample of blood was withdrawn and the catheter was then inserted into either the right or the left (dogs 3, 7, 9, 1-8, 1-9) renal vein. In order to verify the position of the catheter, the extraction of PAH was determined in all instances. Priming and sustaining infusions of PAH were administered and after 30 minutes, arterial and renal venous blood (obtained by allowing the syringe to fill by venous pressure) was withdrawn and analyzed for PAH by the method of Smith (5). The determined extraction of PAH varied from 64 to 88 per cent[1] at arterial PAH concentrations below 2.5 mg. per cent. No effort was made to prevent the diffusion of PAH from the cells of renal venous blood (6). Hence the true extraction exceeded the determined values by approximately 5 per cent (6). Studies in which the extraction ratios were below 64 per cent were discarded. No difference was noted between the extraction of PAH by the right and left kidneys.

Samples of arterial and renal venous blood were withdrawn for assay for free and total amino nitrogen content and an intravenous priming infusion was administered containing 1 to 2 gm. of dog hemoglobin as an 8 to 10 per cent saline solution, prepared according to the method of Amberson and his associates (7). This was followed by a sustaining infusion of 0.5 to 1 per cent hemoglobin administered over a 2 hour period at a rate of 1 to 2 mg. per minute. Frank hemoglobinuria was induced in all studies and plasma hemoglobin concentrations, determined by the method of Evelyn and Malloy (8), varied from 250 to 504 mg. per cent. In 16 studies samples of arterial and renal venous blood were withdrawn 1 hour and again at 2 hours following administration of the priming solution of hemoglobin. At 2 hours the catheter was once again inserted into the inferior vena cava below the renal veins and a sample of blood was withdrawn. In 5 dogs samples of arterial, inferior vena caval and renal venous blood were obtained 5 hours and 24 hours after the administration of hemoglobin.

In 11 animals the "free" alpha amino nitrogen content of plasma was determined by a modification of the photometric ninhydrin method of Moore and Stein (9). Protein-free filtrates of plasma were prepared by precipitation with tungstic acid. To each of two 0.5 ml. aliquots of filtrate, 2.0 ml. of 4 per cent ninhydrin in methyl cellosolve was added. The mixture was shaken well, heated for 30 minutes in a vigorously boiling water bath, and allowed to cool. To this mixture 4 ml. of *n*-proponal (1:1) were added and readings were made on a Coleman Jr. spectrophotometer at 570 mμ after allowing 15 minutes for maximum color development. A standard curve was prepared using leucine in concentrations of 3 to 10 γ per cent. Recoveries of known quantities (2 to 6 mg. per cent) of this amino acid from solutions of 6 per cent human serum albumin averaged 102 ± 2.0 per cent of theoretic values (range, 88 to 112 per cent) following precipitation of protein with tungstic acid. The lower values obtained by this method (2.75 ± 0.63 mg. per cent, Table I) than by the gasometric method of Hamilton and Van Slyke (3.39 ± 0.57 mg. per cent, Table II) may be attributed to a lower ninhydrin color yield for many amino acids than for leucine (9). In 5 dogs (1-8, 1-9, 2-1, 2-2, 2-3) the total alpha amino nitrogen content of the plasma of renal venous, arterial and inferior vena caval blood was determined by the gasometric ninhydrin–carbon dioxide method of Hamilton and Van Slyke (10) after hydrolysis of plasma filtrates with 12 N hydrochloric acid for 4 hours at 259°C. under a pressure of 20 pounds per square inch. Hydrolysis under these conditions rendered the photometric ninhydrin unsuitable owing to the liberation of ammonia and variable quantities of ninhydrin-reacting material other than α-amino nitrogen. The free α-amino nitrogen content was also determined by the gasometric method in these 5 dogs.

[1] At these figures the arterial–renal venous oxygen difference was 2.5 volumes per cent or less. Below 64 per cent extraction, the A-V oxygen difference increased above 2.5 volumes per cent suggesting contamination by inferior vena caval blood (6).

RESULTS

The results are presented in Tables I and II. The free alpha amino nitrogen content of renal venous blood as determined by the photometric ninhydrin method did not differ significantly from that of arterial or inferior vena caval blood during the control observations or after the infusion of hemoglobin for a period of 1 to 2 hours (Table I). There was no difference in the free amino

TABLE I

*"Free" Alpha Amino Nitrogen Levels before and during Hemoglobin Infusions in Dogs as Determined by the Photometric Ninhydrin Method**

Dog	Control			Hemoglobin infusion		
	Artery	Renal vein	Vena cava	Artery	Renal vein	Vena cava
	mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
1	3.00	2.80	3.13	2.73	2.73	3.13
2	3.55	3.75	—	3.32	3.34	3.28
3	3.11	2.90	2.90	2.40	2.26	2.50
4	2.15	2.15	2.18	1.97	2.22	2.06
5	2.13	2.13	2.06	2.22	2.00	3.08
7	2.47	2.87	2.68	2.60	2.61	2.71
9	1.42	2.10	2.34	2.18	2.08	2.10
1-1	3.02	3.18	3.38	2.80	2.74	2.77
1-2	2.47	2.59	2.71	2.42	2.39	2.22
1-5	2.46	2.59	2.59	2.49	2.61	2.50
1-7	3.33	3.33	3.36	2.94	2.87	2.65
Mean	2.75	2.76	2.73	2.55	2.54	2.64
S.D.	±0.63	±0.52	±0.46	±0.39	±0.39	±0.41

* The control values represent single determinations; the values tabulated under hemoglobin infusion are averages of determinations made at 1 and 2 hours after beginning the infusion. In dogs 3, 7, and 9 samples of blood were obtained from the left renal vein; samples were taken from the right kidney in the remainder.

nitrogen content of plasma obtained from the left (dogs 3, 7, and 9) and right renal veins.

In the studies in which free and total alpha amino nitrogen were determined by the gasometric ninhydrin method there was no demonstrable difference between arterial, renal venous, and inferior vena caval blood in either the control periods or during the infusion of hemoglobin (Table II). Additional samples obtained from these animals 5 and 24 hours after administration of hemoglobin did not differ significantly from those values shown in Table II. There was no difference between right and left (dogs 1–8 and 1–9) renal venous blood.

The total alpha amino nitrogen content of inferior vena caval blood was higher than arterial blood both before and following hemoglobin infusions,

TABLE II

*"Free" and Total Alpha Amino Nitrogen Levels as Determined by the Gasometric Ninhydrin Method**

Dog	Control			Hemoglobin infusion		
	Artery	Renal vein	Inferior vena cava	Artery	Renal vein	Inferior vena cava
	mg. %	*mg %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
	Free amino nitrogen					
1-8	3.32	3.17	3.79	2.53	2.57	3.10
1-9	4.06	3.92	3.98	4.11	3.81	4.21
2-1	2.82	2.96	3.02	2.91	3.00	3.00
2-2	3.80	3.70	3.52	3.53	3.91	4.02
2-3	2.96	3.01	3.42	3.21	2.85	3.61
Mean	3.39	3.36	3.55	3.26	3.23	3.59
S.D.	±0.57	±0.43	±0.37	±0.59	±0.61	±0.54
	Total amino nitrogen					
1-8	4.57	3.05	4.93	4.27	3.66	4.82
1-9	5.01	4.92	5.61	4.92	4.86	5.75
2-1	3.50	4.00	4.22	3.80	4.00	3.76
2-2	5.71	5.21	5.50	5.06	5.00	5.21
2-3	4.76	4.50	5.13	4.92	4.44	5.27
Mean	4.71	4.34	5.08	4.59	4.39	4.97
S.D.	±0.80	±0.57	±0.55	±0.54	±0.43	±0.75

* The control values represent single determinations; the values tabulated under hemoglobin infusion are averages of determinations made at 1 and 2 hours after beginning the infusion. Samples of blood were taken from the left renal vein in dogs 1-8 and 1-9 and from the right in dogs 2-1, 2-2, and 2-3.

but the difference between the means was not significant ($t = 2.30$; $p < 0.10 > 0.05$).

DISCUSSION

Addis in 1949 (11) suggested that degradation and catabolism of reabsorbed protein might occur within renal tubular cells with consequent elevation of renal-venous amino nitrogen levels above the arterial concentration as nitrogenous end-products of this metabolic activity entered the circulation. Subsequently Oliver and his associates (12, 13) demonstrated histologically a dynamic component of protein droplet formation within tubular cells suggesting metabolic transformation of reabsorbed protein. In 1955 Eliasch and

his coworkers (3) found that the free amino acid and polypeptide content of renal venous blood of rabbits exceeded by small amounts the concentration of these constituents in arterial blood and suggested that this difference might arise as a result of metabolism of either structural or reabsorbed protein. These workers favored the latter hypothesis although attention was called to the lack of evidence in support of this view.

On the basis of this study Eliasch and his coworkers suggested that 13.6 mg. of protein per 100 ml. of glomerular filtrate might be reabsorbed and catabolized in rabbits. Other observers have suggested that protein may be abstracted from the glomerular filtrate of mammals in amounts up to 25 mg. per 100 ml. of filtrate under normal circumstances (1). Reabsorption and catabolism to amino nitrogen of this amount of protein in the dog would elevate the total alpha amino nitrogen content of renal venous blood above arterial levels by approximately 1.0 mg. per cent, assuming a renal plasma flow and glomerular filtration rate of 100 and 25 ml. per minute, respectively, in a single kidney, and a nitrogen content of reabsorbed protein of 16 per cent. (The small amount of amino acids and peptides lost in the urine would not affect these calculations appreciably.) The failure of the present study to demonstrate an arteriovenous difference of this magnitude suggests that protein is not reabsorbed in this amount under the conditions employed in this study, or that, if reabsorbed, pathways other than degradation to polypeptides and amino acids are available for disposal of this protein from tubular cells.

The failure to demonstrate an elevation of renal venous amino nitrogen content following the administration of 3 to 6 gm. of hemoglobin may be ascribed to similar factors. Assuming complete breakdown of reabsorbed hemoglobin to amino nitrogen, at a renal plasma flow of 100 ml. per minute and a maximum transfer rate of hemoglobin by tubular cells of 1.0 mg. per minute per kidney (2), the total alpha amino nitrogen content of renal venous blood would be elevated by only 0.2 mg. per cent, a quantity too small to measure accurately. Since the methods employed in the present study would not detect accurately an arteriovenous difference of less than 0.5 mg. per cent the possibility is not excluded that protein is abstracted from glomerular filtrate and metabolized, although the failure to detect a significant difference between arterial and renal venous blood at a time when both plasma protein and hemoglobin might be expected to be undergoing metabolic degradation to amino nitrogen suggests that the quantity which may be involved is small. Moreover, a complete study of renal protein and amino acid metabolism is not obtained by a determination of the free and total alpha amino nitrogen content alone since differences between arterial and renal venous blood with respect to the concentrations of individual amino acids and polypeptides may not be reflected in these measurements.

SUMMARY

No significant difference was demonstrated in the free and total alpha amino nitrogen content of arterial, renal venous, and inferior vena caval blood of normal anesthetized dogs during fasting and following the administration of 3 to 6 gm. of hemoglobin. These results do not provide evidence of renal protein reabsorption and metabolism in the dog under the conditions employed in this study.

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