reduction and by depressed gamma globulin peak in gel electrophoretic patterns.

This system, as modified in the present work by eliminating the cumbersome tube feeding technique, will be very useful in further studies of the early events occurring after immunization and of the mechanism of action of β-3-TA.

1. La Via, M. F., Uriu, S. A., Barber, N. D., and Warren, A. E., Proc. Soc. Exptl. Biol. Med. **104**, 562 (1960).

2. La Via, M. F., Proc. Soc. Exptl. Biol. Med. **114**, 133 (1963).

3. La Via, M. F., Hammond, W. S., Iglewski, B. H., Vatter, A. E., Bean, M., and Northup, P. V., *In* "The Reticuloendothelial System and Atherosclerosis," Plenum Press, New York (1967).

4. Sterzl, J., Folia Microbiol. **4**, 91 (1959).

5. Hruban, Z. and Wissler, R. W., Cancer Res. **20**, 1530 (1960).

6. Cannon, P. R., Wissler, R. W., Woolridge, R. L., and Benditt, E. P., Ann. Surg. **120**, 514 (1944).

7. Mach, B. and Vassalli, P., Proc. Nat. Acad. Sci. U.S. **54**, 975 (1965).

8. Davis, B. J., Ann. N. Y. Acad. Sci. **121**, 404 (1964).

9. Hotham-Iglewski B. and Ludwig, E. H., Cancer Res. **27**, 181 (1967).

Received Feb. 6, 1969. P.S.E.B.M., 1969, Vol. 131.

Influence of Uranium-Induced Renal Injury on Flow and Composition of Renal Lymph (34005)

S. J. LeBrie
(Introduced by R. C. Little)

Department of Physiology, College of Medicine, The Ohio State University, Columbus, Ohio 43210; and Tulane University, New Orleans, Louisiana 70112

This laboratory has utilized renal lymph flow and composition as an index of changes occurring in renal interstitial fluid volume and composition (1–3). Renal lymph concentrations of dextran fractions of known molecular weight have also given information on renal peritubular capillary "pore size" and permeability (4). Several years ago we made a preliminary report on the changes occurring in lymph flow and composition after uranium nitrate induced tubular necrosis (5). Recurrence of interest in the mechanism of oliguria or anuria which occurs in acute tubular necrosis (6), as well as the introduction of micropuncture approaches to the problem (7, 8) rekindled our interest in this subject and prompted the present report.

Many investigators have studied the histological and physiological changes which occur in the kidney after uranium intoxication. MacNider, the most prolific of these, reviewed his own work in the Harvey Lectures of 1929 (9). He reported that the epithelium of the convoluted tubules showed the selective action of uranium by developing edema and necrosis which varied in degree with the duration of the injury. The main site of injury was the proximal tubule. The cells of the descending limb of Henle's loop rarely showed evidence of damage. He also found that glomerular changes, unlike those of the tubules, were neither marked nor progressive. The changes in glomeruli are purely degenerative during the acute stages of the process and both degenerative and proliferative phenomena are seen in later stages. Glomerular basement membrane damage is seen in later stages and probably accounts for the observed proteinuria.

The histological changes found in the present study are similar to those reported by others (see "Appendix"). The primary damage at the time of the experiment appeared to be localized in the terminal portion of the proximal tubule and the pars recta. Apparently necrosis and regeneration occurred earlier in the ascending limb and distal tubule. The present results suggest that changes in

tubular permeability produced by uranium injury are reflected by changes in flow and composition of renal lymph.

Methods. Tubular injury was produced in 10 healthy dogs by subcutaneous injection of uranium nitrate (4 mg/kg). Nineteen animals served as controls. Because of the fact that we have control dogs as well as control periods for the injured and uninjured groups, the control dogs are designated "normal" in the text. The quotation marks are in recognition of the fact that all lymph studies are carried out on a selected group of animals, *i.e.*, on the basis of the presence of a lymphatic suitable for cannulation. Both groups were allowed food and water *ad libitum*. Urinary protein loss was determined qualitatively, during a 2–7-day period, with sulfosalicylic acid. Every dog in the uranium group showed a proteinuria ranging from 2 to 4 plus. Water and food consumption progressively diminished and a metabolic acidosis of varying severity developed (CO_2 combining power of the order of 15 meq/liter). No attempt was made to correct the acid–base disturbance.

On the day of the experiment, the animals (control and experimental) were anesthetized by intravenous injection of sodium pentobarbital (25 mg/kg). Both ureters were catheterized at their junction with the bladder. The catheters were positioned within 1–2 in. of the kidney and firmly tied in place. The left kidney was exposed by a flank incision and a single capsular lymphatic was cannulated as previously described. Control samples of lymph, jugular plasma, and urine were obtained. The plasma sample was obtained in the middle of a lymph and urine collection period. At the end of a suitable control period, each animal received 20 ml/kg of a 12.5% mannitol solution in water, at the rate of 10 ml/min. Collection of urine and sampling of lymph and plasma continued throughout the infusion and for a short time after. Creatinine clearances were determined in two of the experimental animals. The method utilized in the above procedures and much of the data from the control group have been previously published (3).

Results. Uranium nitrate administration, 2–7 days before the experimental period, markedly elevated capsular renal lymph flow. Table I shows that mean lymph flow in this group was 41.8 $\pm$ 10.5 μl/min. In a group of normal control animals, under similar experimental conditions, mean lymph flow was 12.4 $\pm$ 2.9 μl/min, a statistically significant difference ($0.025 > p > 0.01$).

There is no apparent correlation between time of uranium administration and change in lymph flow. However, histological evidence does suggest some regeneration in dogs studied at longer intervals after uranium injury (see "Appendix"). In this frame of reference it may be significant that dogs nos. 4 and 5 were studied only 2 days after uranium administration and exhibited the smallest lymph flows.

The most marked change that occurred after uranium injury was a decrease in lymph protein concentration. Table I shows that in a group of 19 "normal" dogs the mean RL/P (renal lymph/plasma) protein ratio was 0.355 $\pm$ 0.55. Uranium injury significantly reduced this ratio to 0.048 $\pm$ 0.020 ($p < 0.001$).

Urine flow and urine osmolality were also reduced in the uranium-treated dogs. The mean U/P osmolality ratio in this group was 1.00 $\pm$ 0.02. Hydrated normal animals in this laboratory usually have osmolality ratios of 4–5, when urine flows are in this low range (Table I).

Effect of mannitol. The effects of mannitol infusion on lymph flow and composition in "normal" dogs has been reported previously (3). A summary of these data appears in Table I, which indicates that a statistically significant 4.5-fold increase in lymph flow occurred. When mannitol infusion was superimposed on uranium injury, an 8-fold increase in lymph flow occurred. It is noteworthy that renal lymph flow increased with mannitol in every animal in the uranium group. Therefore, this increase is highly significant ($p < 0.01$). Although mannitol loading increased lymph flow more in the uranium group as compared to normal animals, *i.e.*, 8-fold vs. 4.5-fold increase, this difference was not statistically significant ($p < 0.1$).

TABLE I. Renal Lymph and Urine Flow in Normal[a] and Uranium Injured Dogs (left kidney).

	Control						Mannitol loading					
	Lymph			Urine			Lymph			Urine		
		RL/P						RL/P				
Dog no.	Flow (μl/min)	Protein	Creatinine	Flow (ml/min)	Na excretion (μeq/min)	U/P osm. press.	Flow (μl/min)	Protein	Creatinine	Flow (ml/min)	Na excretion (μeq/min)	U/P osm. press.
Normal												
Mean	12.4	0.355	0.785	0.216	8.48	4.74	65.8	0.243	0.897	3.90	234.0	1.25
SEM	±2.9	±0.055	±0.053	±0.045	±2.25		±25.3	±0.045	±0.057	±0.44	±98.4	
(*N*)	(19)	(14)	(10)	(19)	(9)	(2)	(19)	(14)	(10)	(19)	(8)	(2)
				Control vs diuresis			$p<.01$[b]	$p<.01$	$p<.01$[b]	$p<.01$[b]	$p<.01$[b]	
Uranium												
1	72.5	0.188		0.028	3.3	1.01	680.0	0.129		0.037	5.2	0.98
2	9.0	0.005		0.004	0.3	0.88	17.0	0.002		0.019	1.5	1.05
3	86.9	0.022		0.167	10.5	0.96	1800.0	0.034		0.769	58.2	—
4	2.6	—	1.26	0.020	0.6	—	4.9	—	1.62	0.158	4.0	—
5[c]	2.3	—		0.003	0.4	—	30.7	—		0.017	2.9	—
6[c]	56.5	0		0.111	3.1	1.03	131.5	0.053		0.454	13.9	0.98
7	60.0	0.029	1.05	0.210	7.9	1.04	490.0	0.026	1.19	1.460	89.9	0.98
8	55.0	0.080		0.018	1.0	1.05	60.0	0.047		0.215	7.3	0.92
9	5.0	0.022		0.230	9.0	1.05	7.0	0		0.640	20.6	1.07
10	68.5	0.039		0.002	0.2	1.00	227.0	0.053		0.013	1.8	—
Mean	41.8	0.048	1.16	0.079	3.6	1.00	344.8	0.043	1.41	0.378	20.5	0.99
SEM	±10.5	±0.020		±0.028	±1.3	±0.02	±177.2	±0.014		±0.148	±9.4	±0.02
				Control vs diuresis			$p<.01$[b]	NS		$p<.01$[b]	$p<.01$[b]	NS
Normal vs. uranium	$.025>p>.01$	$p<.001$		$.025>p>.01$	NS		NS	$p<.001$		$p<.001$	$.05>p>.025$	

[a] Much of the data from normal dogs was published previously (3).

[b] Nonparametric method: The sign test (Wilcoxon rank sum test) is a qualitative test of significance. It is based on the assumption that in the absence of an experimental effect, the experimental value will by chance alone, be greater than the control value one-half of the time. This test is advantageous when there are large differences between individual control values, as is the case with the total protein concentration of lymph or lymph flow measurements.

[c] Mannitol infused at rate 5 ml/min.

No significant difference was observed between RL/P protein ratios in the control and during the mannitol infusion periods, in the uranium group. The protein concentration of lymph remained at about 4% of that in plasma. These results are different from those obtained in the "normal" group during the same mannitol loading, where a significant fall in the protein ratio occurred. However, it must be borne in mind that in this "normal" group renal lymph always contained some 24% of the plasma protein concentration, while in the injured group, lymph only contained trace amounts of protein (4%). Further substantiation of the RL/P protein data is the increase in water content of renal lymph in the uranium group. In three uranium treated dogs lymph water content was 98% while plasma contained 91% water. In nine "normal" dogs mean renal lymph water content, estimated from weight change after drying, was 94.5% while plasma was 91% water. Mannitol loading did not significantly change lymph and plasma water content in uranium-poisoned animals. In "normal" dogs however, lymph water content increased to 97.5% with no change in plasma water.

Although mannitol infusion increased urine flow ($p<0.01$) in the injured group, the maximal mean flow was only 0.378 ml/min as compared to 0.079 ml/min for uranium-poisoned animals without mannitol. This 5-fold increase is to be compared to the 20-fold increase seen in the "normal" group after the same mannitol loading.

Mannitol infusion increased sodium excretion in every dog in the uranium series. The 5-fold increase in sodium excretion is apparently due to the change in urine flow rather than an increase in the sodium concentration of the urine. This is also seen in the U/P osmolality ratio of 0.99 which is not significantly different from the control or premannitol osmolality ratio. It is difficult to assess the degree of renal damage present from urine electrolyte and osmotic pressure data. In general, most of the animals had urine Na, K, and Cl values which were remarkably similar to the plasma concentrations of these electrolytes. Three dogs, nos. 2, 4, and 9 exhibited urine Na and K values which were approximately equal (order of 50 meq/liter). In these animals, Na and K ions were essentially balanced by Cl ions.

Creatinine clearances were determined in two animals after uranium injury. In dog no. 7, clearance of creatinine was 1.6 ml/min during control periods and 3.9 ml/min during mannitol infusion. Similarly obtained creatinine clearances in dog no. 8 were 0.08 and 0.51 ml/min for these periods. It should be noted, that these values are reported as clearance and not glomerular filtration rate.

In a group of 10 normal animals, shown in Table I, mean RL/P creatinine ratio during control periods was 0.785 ± 0.053. This value was increased to 0.897 ± 0.057 during mannitol infusion ($p<0.01$). In two dogs in which RL/P creatinine ratios were obtained after uranium injury the values during the control period were 1.04 and 1.26. During mannitol infusion in these dogs, RL/P creatinine ratios were elevated to 1.19 and 1.62, respectively.

Discussion. These data indicate that uranium nitrate injury to the kidney increased renal lymph flow and markedly reduced the protein concentration of lymph. Hayman *et al.* in 1939 (10) reported that uranium poisoning in dogs resulted in reduction in inulin, creatinine, and urea clearance, although renal blood flow, measured directly, was not reduced. These authors concluded that the reduction in clearances was due to back diffusion of the test substances through injured tubular epithelium. It followed from this that inulin clearance was in no way related to GFR in the damaged kidney.

Eisner *et al.* (6) recently studied the distribution volume of inulin and sodium in the kidney, during anuria produced by uranium. These workers concluded that during anuria the distribution volume of inulin was greater than in the normal dog kidney, and nearly identical to the distribution volume of sodium. These findings suggest that inulin has access to and egress from the tubular lumen. Similar conclusions were reached by Biber *et al.* (7) from micropuncture studies of potassium dichromate damaged kidneys. Bank *et*

al. (8) using micropuncture techniques found that in mercury poisoning, anuria can occur in the presence of normal glomerular filtration rate. Since inulin clearance was normal at the beginning of the proximal tubule, but fell by 60% in the late proximal convolution it was concluded that the proximal tubule had become permeable to inulin.

The data presented in the present paper indicate that under conditions of tubular damage the renal lymphatic system may be of paramount importance in draining cortical interstitial fluid. The low protein concentration of lymph in the uranium treated group is probably a reflection of tubular leakage. This is also indicated by the increase in water content of renal lymph in the uranium group. Thus, uranium injury appears to increase the water content of renal lymph to approximately the same levels as does mannitol loading in "normal" dogs.

The RL/P creatinine ratio of 0.79 in "normal" animals is probably a reflection of diffusion equilibrium between peritubular blood creatinine concentration and renal lymph. Assuming a filtration fraction of about 20%, and the return of most of the filtered water without creatinine to the blood, we would expect peritubular blood to contain about 80% of systemic blood creatinine concentration. If this concentration of creatinine then reaches diffusion equilibrium with renal lymph we would expect renal lymph to contain about 80% of systemic blood creatinine (RL/P creatinine = 0.79). The fact that RL/P creatinine ratio increases to 0.89 during mannitol loading supports this conclusion, since we have reported elsewhere (3) that filtration fraction fell in these dogs during mannitol loading. The finding that RL/P creatinine ratio was greater than 1.0 in the two uranium-injured dogs studied, suggests that creatinine was being added to the lymph from some source other than the peritubular blood. The most likely source is the proximal tubule where early undamaged segments would be expected to concentrate creatinine so that fluid leaking from more distal segments of the proximal convolution would have a creatinine concentration higher than that of plasma. The increase in the RL/P creatinine ratio during the mannitol loading is ascribed to a decrease in filtration fraction as in "normal" dogs. This decrease in filtration fraction observed in "normal" dogs was associated with a fall or no change in GFR and an increase in total blood flow. The medullary component of the blood flow increase appeared to be of primary importance in determining the increase in lymph flow observed (3). Unfortunately, no definite conclusion can be drawn in regard to a medullary component from these data.

Assuming that renal lymph is a fair index of interstitial fluid, these data indicate that renal interstitial protein concentration is markedly reduced when tubular leakage occurs. Since plasma protein was not reduced (mean value 7.2 g/100 ml) and since some degree of glomerular filtration was probably still maintained, protein concentration of the peritubular blood would be expected to remain high. The interrelationship between Starling forces would therefore suggest a marked elevation in the inward or reabsorptive component.

In order to ascertain the function of the lymphatics, let us assume absence of lymph outflow. Under these conditions interstitial fluid inflow into the capillaries would be expected to reduce the oncotic pressure gradient which would consequently limit further inflow of fluid. As new blood with a higher oncotic pressure flows into the capillaries this limiting process is repeated over and over again. If a point is reached where proximal tubular leakage occurs faster than capillary reabsorption, interstitial edema would occur. This would initiate a cycle whereby increased interstitial pressures reduce venous outflow by compression and consequently increase capillary hydrostatic pressure. The Starling forces would now be shifted so that reabsorption is further reduced.

Because of the presence of the lymphatics and outflow of large volumes of low oncotic pressure lymph, these changes are minimized. Thus, the oncotic pressure of peritubular blood is diluted proportionately less. This assures maintenance of a high oncotic pressure

gradient between the capillaries and the interstitium, prevents edema formation and probably is the main function of the renal lymphatic system.

Summary. Uranium nephropathy resulted in a 3-fold increase in renal lymph flow as compared to the flow in control, untreated animals. Normal animals showed a significant 4.5-fold increase in lymph flow during mannitol loading while uranium treated animals showed a significant 8-fold increase. The difference between groups (4.5 vs. 8-fold change) was not significant, however. All animals with uranium nephropathy showed a significant proteinuria at the time of the experiment as well as a significant reduction in lymph protein concentration. Prediuresis levels of urine flow were higher in untreated as compared to treated animals. Mannitol infusion was accompanied by an average increase in urine flow of about 20-fold in normal control animals. Animals with uranium nephropathy showed about a 5-fold increase in urine flow. These data appear to be consistent with the histological finding of primary damage to the distal segment of the proximal tubule resulting in leakage of fluid and electrolytes. This leakage of proximal tubular fluid results in a decrease in protein concentration of cortical interstitial fluid and an increase in interstitial volume. These are reflected as an increase in renal lymph flow and a decrease in lymph protein concentration.

Appendix. We are grateful to Dr. W. H. Sternberg of the Department of Pathology, Tulane University for examining the kidneys of the experimental dogs and for the following report:

"Glomerular changes in general are mild and presumably secondary. These changes consist of focal increase in cellularity of tufts and hyperemia. I would classify the glomerular changes as a mild focal reactive glomerulitis.

In most kidneys the first portion of the proximal convoluted tubules are relatively spared, *i.e.*, minor degenerative changes (vacuolization of the cytoplasm, etc.) but no frank necrosis. The terminal portions of the proximal convoluted tubules are severely involved, particularly the pars recta. These show massive necrosis and loss of nuclei. Lower down, that is, in the juxtamedullary region, these necrotic tubules show replacement by basophilic regeneration epithelium due perhaps to the earlier occurrence of necrosis here, with time for regeneration to be manifested.

The thick limbs of Henle (ascending) show focal and less severe necrosis in the outer medullary zone and many casts. However, as the thick limbs ascend they appear to show replacement of the epithelium by flattened basophilic regenerated epithelial cells, which process continues on into the distal convoluted tubules. Virtually all distal convoluted tubules are dilated, and their characteristic epithelium replaced by flattened regenerated cells. Presumably necrosis has occurred early here, the necrotic cells have been eliminated and replaced by characteristic regenerating epithelium with many mitoses. The macula densa of the juxtaglomerular apparatus appears spared, however. There is considerable focal inflammation of a subacute and chronic type in the interstitial tissue. This varies from animal to animal."

1. LeBrie, S. J. and Mayerson, H. S., Proc. Soc. Exptl. Biol. Med. **100**, 378 (1959).

2. LeBrie, S. J. and Mayerson, H. S., Am. J. Physiol. **198**, 1037 (1960).

3. LeBrie, S. J., Am. J. Physiol. **215**, 116 (1968).

4. LeBrie, S. J., Am. J. Physiol. **213**, 1225 (1967).

5. LeBrie, S. J. and Mayerson, H. S., Physiologist **3**, 102 (1960).

6. Eisner, G. M., Slotkoff, L. M., and Lilienfield, L. S., Am. J. Physiol. **214**, 929 (1968).

7. Biber, T. U. L., Mylle, M., and Gottschalk, C. W., Proc. Intern. Congr. Nephrol., 2nd, Prague, 21 (1963).

8. Bank, N., Metz, B. F., and Aynedjian, H. S., J. Clin. Invest. **46**, 695 (1967).

9. MacNider, W. DeB., Harvey Lectures, Ser. **24**, 82 (1928–29).

10. Hayman, J. M., Shumway, N. P., Dumke, P., and Miller, M., J. Clin. Invest. **18**, 195 (1939).

Received Mar. 20, 1969. P.S.E.B.M., 1969, Vol. 131.