Thus our findings lend further support to present concepts of the role of the thymus in the immune response. Their implications as to the pathogenesis of leukemia are less clear but yet quite provocative. It may be that presentation of the leukemia-inducing virus to the susceptible cell, the immature thymocyte(11,12) leads to its incorporation into the genome of the cell. This incorporation in turn may compromise the normal development of the thymus cells and result in the observed functional impairment long before the appearance of obvious malignancy.

Further studies are being carried out to determine more precisely the degree of functional impairment of thymic function with regard to graft rejection across stronger histocompatibility barriers and graft-*vs*-host reactivity.

Summary. Six-week-old C3H mice injected neonatally with Gross passage A leukemia virus were found to be unable to reject skin grafts across a weak histocompatibility barrier. The similarity of the defects in the immune mechanisms of these mice to those of the neonatally thymectomized mice suggests that the establishment of the oncogenic agent in the neonatal thymus results in marked functional impairment long before histologic changes are apparent.

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Micropuncture Study of Water Reabsorption and PAH Secretion in Urea Diuresis in Rats.* (30324)

THOMAS U. L. BIBER,† MARGARET MYLLE, WILLIAM E. LASSITER‡ AND CARL W. GOTTSCHALK§

Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, N.C.

Platt(1) proposed that in advanced renal failure urine is formed by a reduced number of nephrons which function normally, but under the condition of osmotic diuresis due to an increased urea load. In evaluation of this hypothesis, it seemed important to examine directly water reabsorption during urea diuresis in the normal animal. The limited micropuncture studies available in urea diuresis(2) indicate lower fractional water reabsorption between glomerulus and distal puncture sites than in nondiuretic animals, and a small concentration gradient for sodium across the proximal tubular epithelium. In the present study an attempt was made to obtain more definitive data about fractional water and solute reabsorption in the different parts of the nephron in this type of osmotic diuresis. In addition, p-aminohippuric acid

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† Advanced Research Fellow, Am. Heart Assn.

‡ Established Investigator, Am. Heart Assn., and Markle Scholar in Academic Medicine.

§ Career Investigator, Am. Heart Assn.

(PAH) was measured to determine renal plasma flow (RPF) and to localize its site of secretion in the nephron.

Methods. Six male rats of the Wistar strain weighing 190-330 g were anesthetized by intraperitoneal injection of pentobarbital, 50 mg/kg body weight, and tubular fluid was collected by micropuncture from surface convolutions of the exposed left kidney. Urine from the left kidney was collected by a ureteral catheter and the rate of flow doubled for clearance calculations. Techniques for micropuncture and for determination of osmolality and radioactivity were as previously described(3). A priming dose of 60 μc of p-aminohippuric 2-H^3 acid and/or 15 μc inulin-C^{14} was administered intravenously, followed by 150 μc/hr of PAH^3 and/or 25 μc/hr of inulin carboxyl C^{14}, contained in 5 or 10% urea in 0.9% saline infused at the rate of 6 ml/hr. At least 40 minutes was allowed for equilibration before collections were begun. After this time nearly constant plasma levels (less than 10% change between consecutive plasma collections) of PAH^3 and inulin-C^{14} were observed.‖ Before and after each micropuncture aliquots of plasma were taken from the inferior vena cava below the entrance of the renal veins. Samples of plasma were collected from the left renal vein several times during an experiment for determination of PAH and inulin extraction ratios. Renal plasma flow was calculated by the method of Wolf(4), and renal blood flow estimated from RPF and hematocrit determinations. Urea concentration was measured by the method of Skeggs(5). In the *Results* to follow, mean values are given with the standard error of the mean and, in parentheses, the number of determinations (N = $\times$). For determination of the significance the "t" test was used.

Results. 1. *Clearances.* The average urine flow was 49.1 $\pm$ 5.2 (N = 24), and average osmolar clearance was 103 $\pm$ 7 (N = 24) μl/min 100 g body weight (BW). Inulin clearance ranged from 0.54 to 1.04 ml/min 100 g and averaged 0.78 $\pm$ 0.03 ml/min 100 g (N = 24). PAH clearance averaged 1.64 $\pm$ 0.05 ml/min 100 g (N = 19).

2. *Net water reabsorption.* Tubular fluid to plasma (F/P) inulin concentration ratios, corrected for plasma water concentration, for the proximal tubule were, except for the puncture nearest to the glomerulus, between 1.35 and 1.86, and showed no tendency to rise at an increasing distance from the glomerulus (Fig. 1). The ratios averaged 1.53 $\pm$ 0.12 (N = 4) for the first third, and 1.66 $\pm$ 0.06 (N = 11) for the second third of the proximal tubule. In the distal convolution F/P ratios averaged 2.90 $\pm$ 0.32 (N = 7) in the first half of the convolution, and a single value at 62% was 2.60. U/P inulin ratios averaged 22 $\pm$ 3.0 (N = 22). All the proximal samples were isosmotic with vena cava plasma within the error of the method, and all the distal samples were hypo-osmotic, with an average F/P ratio of 0.63 $\pm$ 0.02 (N = 8).

3. *PAH-secretion.* F/P PAH ratios ranged from 1.04 to 4.67 in the proximal tubule, from 5.54 to 15.09 in the distal convolution, and from 31 to 126 in the ureteral urine.

In Fig. 2 the F/P ratios along the nephron have been divided by the F/P inulin ratios to correct for changes in PAH concentration caused by net water movement. PAH/inulin concentration ratios were as low as 0.86 and averaged 1.12 $\pm$ 0.17 (N = 4) for the first third, and 1.53 $\pm$ 0.20 (N = 7) for the second third of the proximal tubule. They averaged 2.75 $\pm$ 0.18 (N = 7) for samples collected from the distal convolution and 2.24 $\pm$ 0.06 (N = 20) for the urine.

4. *Renal circulation.* Renal extraction of PAH was 75.5 $\pm$ 1.0% (N = 12), and of inulin 34.7 $\pm$ 1.9% (N = 12). Renal plasma flow, calculated from PAH clearance and extraction, averaged 2.28 $\pm$ 0.07 ml/min 100 g BW (N = 12), in good agreement with the average value of 2.24 $\pm$ 0.10 ml/min 100 g obtained from calculations based on inulin clearance and extraction. These figures are very close to the average PAH clearance of 2.2 ml/min 100 g in the unanesthetized rat reported by Peters(6). Renal plasma flow, however, was probably higher

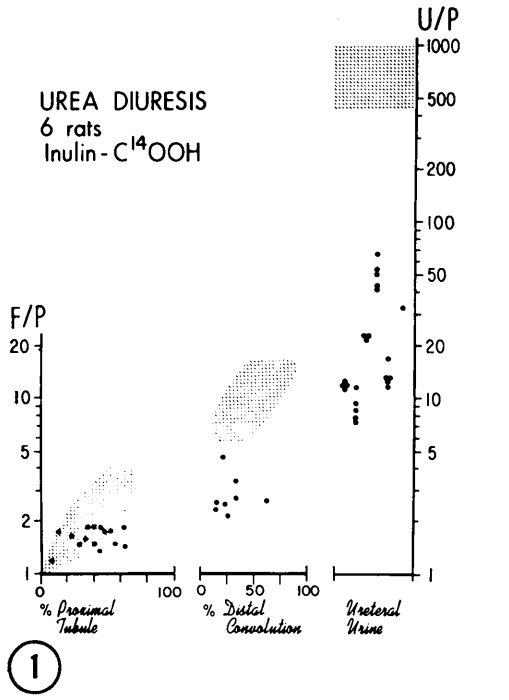
‖ Although not measured in these experiments, calculations based on the specific activity of the PAH solution indicate plasma PAH concentrations were approximately 0.15 to 0.35 mg%.

TABLE I. Results of Analysis of Tubular Fluid, Urine, and Plasma.

Tubule			Urine						Plasma						
											RPF§		RBF‖		
Location*	F/P osm.	F/P PAH	F/P inulin	U/P osm.	U/P PAH	U/P inulin	U/P urea	Volume µl/min 100 g BW‡	C_{in}†	% Extraction PAH	% Extraction Inulin	PAH ml/min 100 g BW‡	Inulin ml/min 100 g BW‡		Osmolality, mOs/kg H_2O	Urea, mg %
								250 g rat—10% urea								
P40	1.03		1.86	1.92		12.4		75.2	.93						352	
P48	.98		1.76	1.86		12.0		87.0	1.04						369	
D62	.66		2.60	1.83		11.8		82.4	.97						378	
P34	1.02		1.58	1.85		11.9		80.0	.95						388	
								320 g rat—10% urea								
P45	1.01		1.82	2.59		32.9		21.6	.71						343	
								190 g rat—10% urea								
P29	1.01	1.30	1.49	1.94	26.7	11.5		59.6	.69	72	34	2.20	2.14		354	
D14	.68	5.59	2.45	1.83	23.4	9.3		70.4	.66						373	
P63	.99	2.12	1.48	1.75	18.9	8.6		81.1	.70	77	28	2.10	2.58		400	
D25	.71	5.54	2.10	1.72	17.4	7.4		92.6	.69						417	
P 8	.99	1.04	1.21	1.73	19.3	7.7	3.5	74.5	.57	76	28	2.09	2.07		445	458
								260 g rat—5% urea								
P55	.99	1.83	1.49	2.51	47.5	22.9		39.1	.90	73	36	2.66	2.41		342	
D15	.58	8.27	2.58	2.54	46.1	21.8		40.5	.88						342	
P44	1.00	1.67	1.35	2.66	46.6	22.2		41.0	.91	80	44	2.56	2.03		342	
				2.88	55.5	28.0	22.9	28.4	.80	73	32	2.32	2.84	3.47	350	156
								330 g rat—5% urea								
P13	1.00	2.00	1.74	3.51	125.8	65.5		15.2	1.00	82	47	2.32	2.13		315	
D33	.52	7.06	3.41	3.35	105.7	53.7		19.0	1.02						316	
P40	.99	1.29	1.49	3.24	86.6	43.4		19.8	.86	71	41	2.53	2.21		319	
D21	.58	15.09	4.61	3.29	85.1	42.4		19.0	.81						323	
P35	1.01	2.85	1.83	3.60	103.4	50.2	20.5	16.8	.84	73	35	2.53	2.57	4.37	328	103
								300 g rat—10% urea								
P53	1.01	3.25	1.74	2.31	43.2	16.7		33.8	.56	77	33	2.06	1.75		348	
D23	.64	6.56	2.49	2.10	32.3	13.1		41.2	.54						358	
P62	1.01	4.67	1.85	2.08	32.6	13.0		42.8	.56	75	33	1.96	1.72		369	
D33	.70	8.56	2.69	2.03	31.3	12.4		47.6	.59						376	
P23	1.01	2.65	1.66	2.01	30.7	11.6	5.7	49.7	.58	77	25	2.14	2.41	3.34	383	232

* Percent length of proximal tubule (P) and distal convolution (D). † Inulin clearance. ‡ Body wt. § Calculated from clearance and extraction ratios of PAH and inulin. ‖ Renal blood flow calculated from RPF (PAH) and hematocrit.

in Peters' experiments, since PAH extraction was most likely not complete. At the low



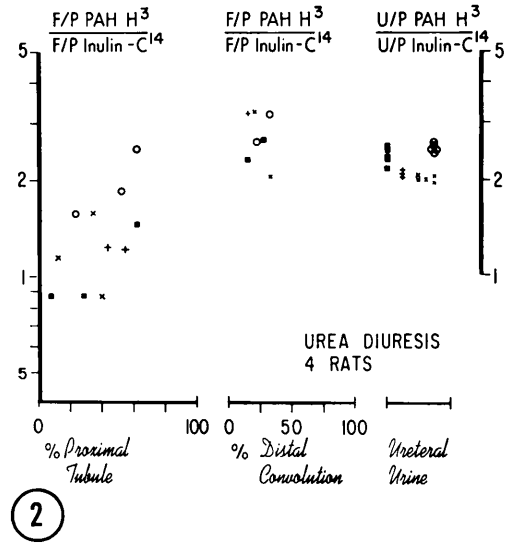


FIG. 1. Inulin fluid/plasma ratios of samples collected from proximal tubule, distal convolution and urine during urea diuresis. Hatched area indicates range of corresponding values in nondiuretic rats(7-9).

FIG. 2. PAH fluid/plasma ratios divided by inulin fluid/plasma ratios of samples collected from proximal tubule, distal convolution and urine during urea diuresis.

hematocrit values of 37% in our experiments, renal blood flow averaged 3.73 ± 0.22 ml/min 100 g (N = 9). Filtration fraction, obtained by dividing the inulin clearance by the renal plasma flow, averaged 0.32 ± 0.01 (N = 12).

5. *Urea.* Plasma osmolality rose at a constant rate during the experiments because of the constant infusion of a hypertonic urea solution and averaged 396 mOs/kg H_2O at the end of the 6 experiments. Plasma urea concentration averaged 237 mg% at the end of the 4 experiments in which it was determined.

Discussion. Water reabsorption. The average F/P inulin ratio in the proximal tubule of nondiuretic rats calculated from available data(7-9) is 1.63 ± 0.12 (N = 14) in the first third, and 2.35 ± 0.11 (N = 35) in the middle third of the tubule. In urea diuresis, fractional water reabsorption in the first third of the proximal tubule (F/P = 1.53) is not significantly different from that in non-diuretic rats. In the second third of the proximal tubule, on the other hand, there is a significant decrease of fractional water reabsorption in urea diuresis when compared with the average value of nondiuretic rats (F/P of 1.66 and 2.35, respectively, $p < 0.005$). This means that in urea diuresis only about 40%, instead of approximately 60%, of the filtered water was reabsorbed in the first half of the proximal tubule. If a normal glomerular filtration rate of 0.6 ml/min 100 g BW is assumed, the total amount of water reabsorbed up to this point of the nephron was nearly as great in urea diuresis as in antidiuresis because of the increased glomerular filtration rate in urea diuresis (0.78 ml/min 100 g).

Wesson and Anslow(10) suggested that during osmotic diuresis osmotically obligated water results in a lowering of the sodium concentration, which may reach a critical value at which sodium reabsorption ceases. Micropuncture studies have demonstrated low tubular sodium concentrations and the dependence of water reabsorption on sodium reabsorption in mannitol diuresis(11,2). It is likely that a similar mechanism also applies in urea diuresis. In addition, a small amount of water will

be osmotically obligated and reabsorbed as a consequence of the back diffusion of urea itself. The increased tubular concentration of urea results, of course, from water reabsorption subsequent to active sodium transport.

In the first half of the distal convolution an average F/P inulin concentration ratio of 8.44 ± 1.26 (N = 9) can be calculated for nondiuretic rats from the data of Lassiter *et al*(7). The corresponding value during urea diuresis is significantly lower (F/P 2.90, $p < 0.005$). The inulin concentration ratios indicate that in urea diuresis 25% of filtered water was reabsorbed between the puncture sites in the late proximal tubule and those in the early distal convolution, similar to the 30% reabsorption observed in antidiuretic rats. Windhager and Giebisch(11) reported that in mannitol diuresis only 10% of the filtered water was reabsorbed between the late proximal tubule and the early distal convolution, and suggested that a limiting concentration gradient for sodium was reached or approached in the pars recta of the proximal tubule, reducing net sodium and water movement across the tubular wall. It is possible that this effect cannot be observed in urea diuresis because of loss of urea out of the tubule, even though the proximal F/P sodium ratios are lower than unity(2).

The inulin concentration ratios indicate that 30% of the filtered water was reabsorbed in urea diuresis in the distal convolution and collecting ducts, in contrast to 10% in the antidiuretic rat. However, in spite of this increased water reabsorption in the later part of the nephron, the lower fractional water reabsorption of the proximal tubule and increased GFR were not fully compensated, as shown by the low U/P inulin ratios.

PAH. These studies demonstrate directly active secretion of PAH by the epithelium of the proximal convolution. Secretion probably continues in the pars recta of the proximal convoluted tubule since the early distal PAH/inulin ratios are somewhat higher than those from the late proximal. There was no evidence of PAH secretion in more distal portions of the nephron. The urinary PAH/inulin ratios were somewhat lower than those from the distal convolution, but this is of questionable significance in respect to loss of PAH from the nephron, since only a vanishingly small percentage of the nephrons were sampled which contribute to the final urine, and these only superficial nephrons. Unpublished studies in this laboratory with the tracer microinjection technique have shown no evidence of loss of PAH from the tubule during osmotic diuresis.

Three proximal PAH/inulin ratios were below unity (0.86, 0.87 and 0.87). This could be due either to leakage of PAH out of the tubule (the negative intratubular potential would favor such a movement since the pK' of PAH is 3.8) or to protein-binding of PAH. We have measured the protein-binding of PAH-H^3 at comparable concentrations of PAH under controlled pH and temperature conditions using 3 different methods (constant equilibrium dialysis with loading of plasma or dialysate(12), ultrafiltration by centrifugation, and ultrafiltration by pressure (13)). The average value for protein-binding was 9.6 ± 0.6% (N = 27) and could explain the low proximal PAH/inulin ratios obtained in this study.

Summary. Inulin and PAH concentrations and osmolalities were determined in samples of tubular fluid collected from surface convolutions of rat kidneys during urea diuresis. F/P inulin ratios averaged 1.6 for the proximal tubule, 2.9 for the distal convolution and 21.7 for the ureteral urine, all significantly lower than in nondiuretic rats. Net PAH secretion appeared to be confined to the proximal tubule and could not be demonstrated in other parts of the nephron.

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The Significance of a Smooth Muscle Component in Hemostasis.* (30325)

W. O. CRUZ
Laboratory of Hematology, Instituto Oswaldo Cruz, Rio de Janeiro, Brasil

Currently accepted theories on the control of bleeding in oozing blood postulate two main but quite distinct mechanisms: formation of blood clots and agglutination of platelets to form a plug in this region. In both instances a mechanical stopper is visualized and hemostasis is interpreted as a phenomenon entirely dependent on blood components, particularly the disintegrative processes concerned with these.

Several observations have been presented (1,2,3,4) which were extremely difficult to resolve in terms of the classical theories noted above. It was then suggested that hemostasis is a function of active metabolic events and that the local area of injury, rather than blood itself, plays the most important role in the control of bleeding.

Methods. The results presented here were carried out by a method developed in our laboratory. A small area of keratinized epidermis in a rat tail is removed with a razor blade and the ensuing bleeding stops in 1 to 2 minutes. When mechanical trauma (such as strong scouring with gauze) is subsequently inflicted on such scarified skin and the tail immersed, in a vertical position, in different fluids at 37°C, the bleeding in each vessel (arteriole or venule) can be observed through a dissecting microscope (30 to 50 × magnification) until hemorrhage completely ceases. Table I shows that this method is quite consistent, the range of bleeding time (B.T.) varying between 40 to 90 seconds (Table I).

Results. Direct visual observation. Bleeding from arterioles never stopped abruptly. There was always a progressive decrease in the diameter of the falling column of blood until a very thin stream was observed just before the end of bleeding.

Repeated mechanical stimulation. When mechanical trauma was applied at about 10-minute intervals, B.T. remained fairly constant for a period of about 2 hours (average for 12 determinations — 56″ with a range of 20″-93″). When, however, the trauma was applied as soon as the bleeding stopped, then, after from 5-10 such stimulations, the scarified skin failed to bleed even though subjected to the most violent mechanical stimulus.

Inhibitors of hemostasis. Inflence of anions and cations. When the saline in the bath was substituted with sodium or potassium salts of non-penetrating anions (HPO_4^{--}, SO_4^{--}, HCO_3^-) at isotonic concentrations, or by salts of slow moving anions (CNS^-, NO_3^-) it was observed that these solutions had a tendency to prolong bleeding. The anions SO_4^{--} and HPO_4^{--} had an especially strong anti-hemostatic action. A strong anti-hemostatic effect with cations was observed when $CaCl_2$ or $MgSO_4$ (10 mM or higher) was tested isotonically in saline.

Inhibitors of cell respiration. Inhibitors of

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