Studies on the Mechanism of Oliguria in a Model of Unilateral Acute Renal Failure

JOHN W. COX, RICHARD W. BAEHLER, HARI SHARMA, THOMAS O'DORISIO, RICHARD W. OSGOOD, JAY H. STEIN, and THOMAS F. FERRIS

From the Departments of Medicine and Pathology, Ohio State University, Columbus, Ohio 43210

ABSTRACT To further evaluate the mechanism of the oliguria of acute renal failure, a model was utilized in which intense and prolonged vasoconstriction produced the unilateral cessation of urine flow. The radioactive microsphere method was used to measure total and regional blood flow before and after the intrarenal infusion of norepinephrine, 0.75 μg/kg/min, for 2 h in the dog. In the control kidney, renal blood flow increased 32% 48 h after norepinephrine in association with a fall in the fractional distribution of flow to the outer cortex. In the experimental kidney, total renal blood flow fell from 190 ml/min before norepinephrine to 116 ml/min at 48 h ($P < 0.025$) with a uniform reduction in cortical blood flow. After the administration of 10% body wt Ringer's solution, there was a marked redistribution of flow to inner cortical nephrons in both the control and experimental kidney. In addition, there was a marked increase in total blood flow in both kidneys. On the experimental side, flow rose to 235 ml/min, a value greater than in either the control period ($P < 0.05$) or at 48 h after norepinephrine ($P < 0.001$). However, in spite of this marked increase in blood flow, there was essentially no urine flow from the experimental kidney. In separate studies, the animals were prepared for micropuncture. In all studies, the surface tubules were collapsed, and there was no evidence of tubular obstruction or leakage of filtrate. Over 99% of the 15-μM spheres were extracted in one pass through the experimental kidney. An analysis of the forces affecting filtration suggested that an alteration in the ultrafiltration coefficient may be responsible, at least in part, for the anuria in this model. In this regard, transmission and scanning electron microscopy revealed a marked abnormality in the epithelial structure of the glomerulus. It is suggested that a decrease in glomerular capillary permeability may be present in this model of acute renal failure.

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INTRODUCTION

The pathogenesis of the oliguria of acute renal failure is not clear. Four mechanisms have been implicated: (*a*) renal vasoconstriction, (*b*) development of an aglomerular shunt pathway, (*c*) tubular obstruction, and (*d*) back leakage of filtrate. Although observations have been made which could be compatible with the latter three possibilities (1–6), the majority of recent data support alterations in renal resistance as the major determinant of diminished filtration in acute renal failure (7–12). Data obtained from micropuncture and hemodynamic studies have led to the view that marked afferent arteriolar constriction decreases effective filtration pressure and glomerular filtration rate. Yet other observations suggest that the responsiveness of the renal vasculature is intact in acute renal failure and that oliguria may persist in spite of a return of blood flow to normal levels (13–14). Ladefoged and Winkler found in studies in man with acute renal failure that the intravenous administration of dihydralazine markedly decreased renal resistance and increased renal blood flow, a response qualitatively like what occurred in normal subjects (13). Jaenike noted that renal blood flow could be returned to normal with saline loading in rats with acute renal failure, while only minor increases in inulin clearance and urine flow occurred (14).

Therefore, it was the purpose of this study to further evaluate the renal hemodynamic changes in a model of acute oliguric renal failure. In considering the possible experimental counterparts of this syndrome, it was decided to utilize a model recently described by Knapp, Hollenberg, Busch, and Abrams in which in-

tense and prolonged renal vasoconstriction produced unilateral oliguric renal failure in the dog (15). The results of these studies indicate that increased renal resistance cannot account for the virtual cessation of filtration and suggest that alterations in glomerular capillary permeability are operative in this model.

METHODS

Female mongrel dogs weighing 15–25 kg were anesthetized with pentobarbital (30–35 mg/kg) and given additional doses as needed during the experiment. A catheter was inserted in the femoral or brachial artery for blood collection and a Goodale-Lubin standard wall catheter was placed in the left ventricle for injection of the microspheres.

Radioactive microspheres (3M Co., Medical Products Div., St. Paul, Minn.) were used to measure regional blood flow in different areas of the renal cortex as well as total renal blood flow. The nuclides used were ^{85}Sr, ^{141}Ce, and ^{51}Cr. A different nuclide was used in each period and the sequence of injection was altered from experiment to experiment. Approximately 500,000 microspheres (15–20 μCi) 15±5 μM in diameter were given with each injection.

The appropriate nuclide to be given was suspended in a 25-ml solution of 10% dextran, injected through the left ventricular catheter in approximately 15 s and then flushed with 5 ml of heparinized saline.

Hemodynamic studies. Unilateral renal ischemia was produced by the intrarenal infusion of norepinephrine, as described by Knapp et al. (15). A selective renal catheter was placed in the orifice of either the left or right renal artery under fluoroscopic guidance from a femoral artery cutdown. No studies were performed in kidneys with more than a single renal artery. The catheter was kept open with an infusion of Ringer's solution at a rate of 1 ml/min. After the animal had been anesthetized and stabilized for 1–2 h, an initial injection of microspheres was given (period I). After the initial injection, norepinephrine, 0.75 μg/kg/min, was given in the renal artery for 90–120 min. During this period of infusion, mean arterial pressure rose in each instance with a mean change of 20±2 mm Hg. At the conclusion of the infusion, the selective catheter was removed and the animal was allowed to awaken and returned to its cage. In five of the studies, the arterial and left ventricular catheters were removed and the arteries were ligated, while in the remaining four studies the lines were tunneled under the skin and left in place for the repeat study. All animals tolerated the procedure well and consumed their daily allotment of food and water in the interval between study periods. 48 h later, the animals were again lightly anesthetized. In the studies in which the arteries had been ligated, the catheters were inserted through an incision proximal to the ligature, while in the other four studies the chronic catheters were utilized. In addition, in this phase of the study, both ureters were cannulated with PE 160 tubing. After stabilization and the recording of arterial pressure, a second injection of microspheres was given (period II). After the second injection of spheres, Ringer's solution was given at a rate of 1.5 ml/kg per min for 45 min and then at a maintenance rate of 5 ml/min above urine flow. 20 min after the maintenance had begun, the third injection of microspheres was given (period III). In four additional studies, the same protocol was utilized except that Ringer's infusion in the renal artery catheter was continued and no norepinephrine was given after the first microsphere injection.

In seven of the studies, total renal blood flow was measured with radioactive microspheres by a method described by Domenech et al. (16). The use of this modified indicator dilution technique obviated the necessity of urine collections or undue manipulation of the kidney in order to obtain renal blood flow. Its validity and correlation with other techniques of measuring renal blood flow has previously been demonstrated (16, 17). In preliminary studies, it was found that the ratio of total renal blood flow between the right and left kidney during hydropenia was 1.00 ±0.02 ($n=7$) with this technique and that the coefficient of variation of replicate injections of two different nuclides was 4% ($n=7$). In addition, the ratio of renal blood flow obtained with microspheres as compared to measurements with a Medicon M-4001 electromagnetic flow meter (Mediconics International Inc., Waco, Tex.) was 0.97±0.03 ($n=4$).

To determine perfusion rate with microspheres, a reference flow rate is required. Reference samples were collected from a PE 260 catheter passed into the abdominal aorta just proximal to the femoral bifurcation or from the brachial artery. The reference sample was then collected in a 30-cm^3 heparinized syringe with a Harvard withdrawal pump (Harvard Apparatus Co., Inc., Millis, Mass.). The pump was started at a withdrawal rate of 24.7 ml/min, 10 s before the injection and continued for 1 min after the administration of the spheres. The reference samples were transferred to counting tubes and the collection syringe was rinsed with Haemo-Sol solution (Meinecke & Co., Inc., Baltimore, Md.). This rinse was added to the counting tube. The tubes were then centrifuged for five min to sediment the microspheres.

At the conclusion of all experiments, the kidneys were removed, weighed, sectioned, and counted by methods previously described (18). To determine regional blood flow, the cortex was divided into four equal zones, which will be called zones I through IV, going from outer to inner cortex, respectively. In the studies in which total renal blood flow was measured, the tissue sections were counted, recombined with the remainder of each kidney and dissolved in 100 ml of concentrated hydrochloric acid for 24–48 h. Triplicate 1-ml aliquots of this solution were then pipetted and counted in the same manner as the tissue sections. The coefficient of variation of these samples was 3%. Similarly, the blood collected during each microsphere injection was counted at the same time.

Extraction studies. In five further studies, a quantification of shunting of the microspheres was obtained by a method recently described from this laboratory (19). Experiments were performed in animals given norepinephrine, as described above, 48 h before study. A 21-guage hooked needle was placed in the orifice of the left renal artery and the left renal vein was catheterized with PE 160 tubing via the ovarian vein. After saline loading, approximately 50,000 radioactive microspheres, 15±5 μm, labeled with ^{85}Sr, were given over 30 s into the renal artery. As the microspheres were being given, the renal vein was clamped distal to the catheter and renal venous blood was continuously aspirated with 50-ml syringes to which small amounts of heparin had been added. Aspiration was continued for 1 min after injection of the spheres.

At the conclusion of each study, the kidney was removed and stripped of perirenal fat and sectioned into 0.3-cm coronal slices and placed in the bottom of a plastic container. The heparinized renal venous blood was placed in the same type of plastic container and the blood was allowed to stand for 2 h.

TABLE I
Summary of Absolute

Kidney Period	Total renal blood flow			Zonal perfusion rate, Zone 1		
	I	II	III	I	II	III
		ml/min			*ml/min/g*	
Experimental kidney $n = 7$	190±20‡	116±11	235±9	6.3±0.3	4.1±0.5	6.1±0.7
P I–II		<0.025			<0.010	
P II–III		<0.001			<0.025	
P I–III		<0.050			NS	
Control kidney	189±22	249±29	313±27	6.7±0.4	7.9±0.8	8.6±0.6
P I–II		<0.010			NS	
P II–III		<0.010			NS	
P I–III		<0.005			<0.01	

* I, Control period; II, 48 h after norepinephrine period; III, saline loading period.
‡ Mean±SEM.

The blood and kidney slices were then counted in a gamma well counter (Packard Model 3002 Tri-Carb Scintillation Spectrometer, Packard Instrument Co., Inc., Downers Groves, Ill.) at 0.510 keV with a special well to accommodate the width of the containers. The distance of the plastic container from the crystal was maintained at 30 cm to keep geometry constant.

Micropuncture studies. In seven studies, the experimental kidney was prepared for micropuncture in a manner previously described from this laboratory (20). A catheter was placed just above the orifice of the renal artery and injections of 1 ml of 5% lissamine green were given in periods II and III to determine whether any dye transversed the renal tubules. In addition, standard techniques were utilized to attempt to collect tubular fluid in 10–15 tubules in each study. In three of these studies, total renal blood flow was also measured with the microsphere method.

Histological studies. In four of the hemodynamic studies, sections were removed and fixed in formaldehyde and then processed for light microscopic examination.

In six additional studies, 48 h after norepinephrine infusion, the animal was prepared in the same manner, and Ringer's solution was given as described previously. A PE 240 catheter was threaded from the femoral artery to a point adjacent to the renal artery orifices. The aorta was then ligated proximally and distally to the renal arteries and the kidneys were perfused at a mean pressure of 120 mm Hg with a 2% phosphate-buffered glutaraldehyde solution at a rate of 250–300 ml/min for 4–5 min. As the kidneys were being fixed, both renal veins were cut to allow drainage of the perfusate. No microspheres were given in these studies.

At the end of the infusion, the kidneys were removed and five representative sections of cortex (10 mm × 3 mm × 1 mm) were cut with razor blades from each kidney. The cutting was performed in such a manner as to include cortex from capsule to medulla and to present a surface suitable for scanning. The specimens were then post-fixed in 1% osmium tetroxide and then washed in phosphate buffer for 1 h. The specimens were dehydrated with graded concentrations of ethanol, amyl acetate in ethanol, and finally amyl acetate alone. The tissue was then dried by the method described by Anderson (21) with carbon dioxide in a critical point dryer (Denton DCP-1, Denton Vacuum Inc., Cherry Hill, N. J.). The dried specimens were mounted on aluminum stubs and coated evenly with gold in a vacuum evaporator (Varian VE-10, Varian Associates, Vacuum Div., Palo Alto, Calif.). The tissue was then examined with a scanning electron microscope with accelerating voltage of 20 keV (Cambridge Mark II, Cambridge Thermionic Corp., Cambridge, Mass.).

Sections were processed for transmission electron microscopy by techniques previously reported (22).

Plasma protein concentration was determined by the Lowry, Rosebrough, Farr, and Randall method (23).

Calculations. Renal blood flow was calculated by the following formula:

$$\frac{\text{Total cpm in blood}}{\text{Blood withdrawal rate (ml/min)}} = \frac{\text{cpm in kidney}}{\text{Renal blood flow (ml/min)}}$$

where cpm is counts per minute of the nuclide.

The methods of calculating the corrected fractional distribution of renal cortical blood flow per cortical zone and the zonal perfusion rate have been described previously (18).

The percentage of microspheres recovered in the renal venous blood was calculated from the following formula:

$$\text{Recovery of microspheres (\%)} = \frac{\text{Renal vein blood cpm}}{\text{Kidney cpm + renal vein blood cpm}}$$

Results are recorded as mean±SEM. Statistical difference was determined by a paired *t* test.

RESULTS

Hemodynamic studies

All the animals tolerated the control procedure well and ate their regular meals in the 48 h interval after

Blood Flow Data

Zonal perfusion rate								
Zone 2			Zone 3			Zone 4		
I	II	III	I	II	III	I	II	III
				ml/min/g				
5.1±0.4	2.9±0.3	5.6±0.7	2.8±0.3	1.9±0.2	6.4±0.8	1.0±0.2	0.7±0.1	4.3±0.5
	<0.01			<0.050			NS	
	<0.05			<0.001			<0.001	
	NS			<0.025			<0.005	
4.9±0.4	7.1±0.4	8.7±1.0	3.1±0.4	4.5±0.4	7.3±1.2	1.6±0.2	2.6±0.3	7.1±1.4
	<0.005			<0.01			<0.025	
	NS			NS			<0.025	
	<0.025			<0.025			<0.010	

norepinephrine. The mean blood urea nitrogen concentration was 17 mg/100 ml at the time of the repeat study. The results of the seven studies in which total renal blood flow was determined are summarized in Table I. Total blood flow in the experimental kidney fell from 190±20 ml/min to 116±11 ml/min 48 h after norepinephrine ($P < 0.025$). After Ringer's loading, flow in the experimental kidney increased from 116±11 to 235±9 ml/min ($P < 0.001$). This latter value was also significantly greater than the value in period I ($P < 0.05$). In contrast, flow in the control kidney increased from 189±22 ml/min in period I to 249±29 ml/min in period II ($P < 0.01$). Ringer's loading increased flow further to 313±27 ml/min ($P < 0.01$). Mean arterial pressure was not significantly different in periods I and II but increased 7±1 mm Hg period III ($P < 0.01$). Plasma protein concentration decreased from 6.4±0.2 to 4.1±0.2 g/100 ml after volume expansion ($P < 0.005$).

During period II, the mean urinary flow rate from the control kidney was 0.3 ml/min while there was no measurable urine from the experimental kidney. During volume expansion, urine flow increased to 6.6±1.2 ml/min on the control side and urinary sodium excretion was 753±81 μeq/min. In contrast, urinary flow was not detectable in six of the nine studies in the experimental kidney. In the remaining experiments, urine did fill the catheter after saline loading, but flow was too low to be accurately measured.

The results of the regional blood flow data are summarized in Tables I and II and Figs. 1 and 2. In the experimental kidney, the fractional distribution of blood flow was unchanged 48 h after the norepinephrine infusion with values of 50, 32, 14, and 4% in period I, and 50, 30, 15, and 5% in period II in zones 1 through 4, respectively. However, after Ringer's loading, the percent of blood flow in outer cortical zone 1 decreased in each of the nine studies with a mean decrease to 37% ($P < 0.001$). There was no significant change in zone 2, while zone 3 increased from 18 to 23% ($P < 0.001$) and zone 4 rose to 11% ($P < 0.001$). Absolute zonal flow fell in all four zones after norepinephrine in parallel with the decrease in total flow. The fall in zone 4 was just below the 0.05 level of significance due to a small increase in one of the seven studies. After Ringer's loading, total blood flow increased 102% while zonal flow increased 51, 93, 236, and 437% in zones 1 through 4, respectively. In addition, in comparison with the values obtained in period I, absolute flow was unchanged in zones 1 and 2 and significantly increased in zones 3 and 4 during Ringer's loading.

Changes were also found in the fractional distribution of flow in the control kidney. The percent of blood flow 48 h after norepinephrine infusion decreased in outer cortical zone 1 from 52 to 46% ($P < 0.025$). There were reciprocal increases of 3, 2, and 1% in zones 2 through 4, respectively. Only the former value reached the level of statistical significance. After Ringer's loading, there was a further fall in the fractional distribution in zone 1 in six of the seven studies with a mean change from 46 to 39%. There was a decrease from 32 to 29% in zone 2 ($P < 0.025$), zone 3 increased slightly from 16 to 19%, and zone 4 rose from 6 to 13% ($P < 0.005$). In comparing the fractional distribution in periods I and III, there was a significant change in zones 1, 3, and 4, indicative of a marked redistribution of flow to inner cortical nephrons. Absolute zonal flow increased in all four zones in period II, though the increase in zone 1 failed to reach statistical significance. After Ringer's loading total blood flow increased 25% while zonal flow

TABLE II

Effect of Unilateral Norepinephrine Infusion and Ringer's

Exp. no.	Experimental kidney Percent distribution to cortical zone											
	Zone 1			Zone 2			Zone 3			Zone 4		
	I*	II	III	I	II	III	I	II	III	I	II	III
1	53‡	57	36	33	29	25	12	10	24	2	3	15
2	50	47	33	32	32	31	14	17	27	4	4	9
3	44	41	38	37	31	30	16	18	21	3	9	11
4	52	52	35	34	27	27	12	16	28	2	5	10
5	61	57	37	25	26	20	11	13	25	3	4	18
6	50	52	43	35	31	31	12	14	19	3	3	7
7	44	50	33	28	27	30	21	18	25	7	5	12
8	50	48	35	36	35	43	12	13	17	2	4	5
9	48	48	36	32	31	25	15	16	23	5	5	16
Mean	50	50	37	32	30	29	14	15	23	4	5	11
SE	1.7	1.6	1.4	1.2	0.9	2.1	1.0	0.8	1.2	0.5	0.6	1.4
P I–II		NS			NS			NS			NS	
II–III		<0.001			NS			<0.001			<0.005	
I–III		<0.001			NS			<0.001			<0.001	

* Period I, control period; II, 48 h after norepinephrine period; III, saline loading period.

‡ All values are the mean of three or more sections in the same kidney.

increased 8, 23, 62, and 173% in zones 1 through 4, respectively. In comparison with the values obtained in period 1, absolute flow increased in all four zones during Ringer's loading.

In the four control experiments, there was no significant alteration in total blood flow or the fractional distribution of flow during the first two periods of study. After volume expansion, there was a marked redistribution of flow to inner cortical nephrons in both kidneys in association with a mean increase in renal blood flow of 47 and 49% in the right and left kidneys, respectively.

Extraction studies

In the five studies in which extraction of the microspheres was evaluated, recovery in renal venous blood ranged from 0.3 to 1.1%. Virtually all of the radioactivity within the kidney was localized to the cortex. On

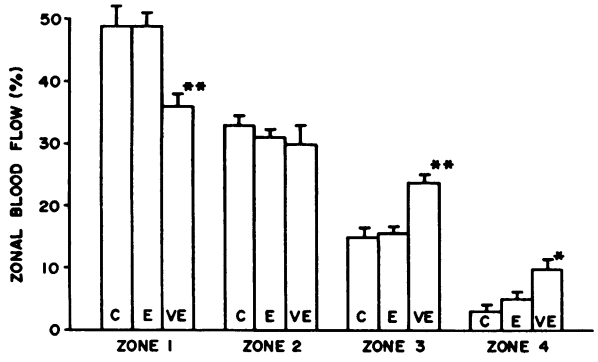


FIGURE 1 The fractional distribution of cortical blood flow in experimental kidney during the three periods of study. $n = 9$; C, control period; E, 48 h after norepinephrine; VE, volume expansion.

* $P < 0.005$.

** $P < 0.001$.

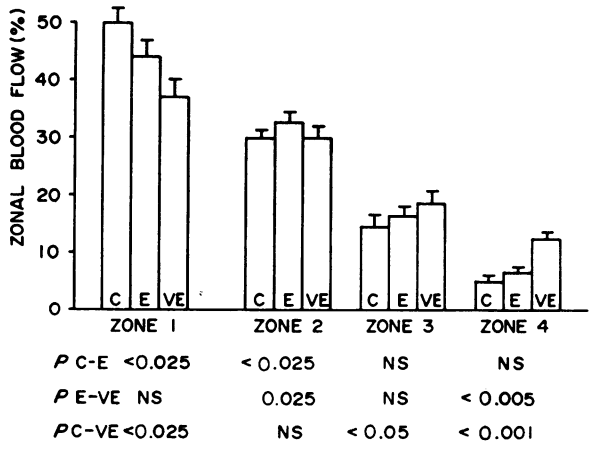


FIGURE 2 The fractional distribution of cortical blood flow in control kidney during the three periods of study. $n = 7$. C, control; E, 48 h after norepinephrine; VE, volume expansion.

Loading on Percent Distribution of Blood Flow in Renal Cortex

Control kidney Percent distribution to cortical zone											
Zone 1			Zone 2			Zone 3			Zone 4		
I	II	III	I	II	III	I	II	III	I	II	III
60	56	42	27	30	26	10	11	18	3	3	14
54	45	40	27	33	28	14	17	19	5	5	13
51	39	42	35	37	34	11	17	15	3	7	9
53	48	29	29	29	30	14	16	24	4	7	17
52	52	49	29	30	26	14	13	13	5	5	12
42	38	33	29	33	29	22	21	23	7	8	15
39	31	30	33	38	40	19	20	20	9	10	10
52	46	39	29	32	29	14	16	19	5	6	13
2.4	2.9	2.9	1.2	1.2	1.2	1.7	1.4	1.7	0.6	0.7	1.1
	<0.025			<0.025			NS			NS	
	NS			<0.025			NS			<0.005	
	<0.025			NS			<0.05			<0.001	

histologic evaluation, the spheres were exclusively found in the cortex within the glomerular circulation.

Micropuncture studies

The experimental kidney was usually somewhat softer than normal and the capsule was not tightly adherent to the cortical tissue. In each study, all surface tubules appeared collapsed on initial inspection. This was confirmed by the failure to obtain any tubular fluid on multiple punctures in each study. Before volume expansion, the peritubular capillary flow rate appeared sluggish. After Ringer's loading, there was an obvious increase in capillary flow rate, which then appeared normal, but the tubules remained collapsed and no tubular fluid could be collected. In both periods, after the injection of lissamine green, the dye appeared in the peritubular capillaries but none was seen to enter the tubules. In three of these studies, total blood flow was measured and decreased from 175 to 110 ml/min after norepinephrine. In addition, after volume expansion, flow rose in each study to a higher level than in the control period, the mean value being 231 ml/min.

Histologic studies

Light microscopy. Morphology of the experimental kidney showed widespread tubular necrosis. The necrosis mainly involved the proximal convoluted tubules. The involved tubules were filled with cytoplasmic debris of epithelial cells (Fig. 3). The nuclei of the tubules showed chromatolysis, pyknosis, and karyorrhexis. Mild intersitial edema was present. The glomerular capillaries were dilated and contained erythrocytes. The glomerular capillary basement membrane was thin, and few, if any, podocytes were discernible.

Transmission electron microscopy. The glomerulus of the control kidney demonstrated a normal podocyte structure with well-formed foot processes resting on the basement membrane. Very few villi were seen coming off the podocytes. The glomerulus from the experimental animals showed slight dilatation of the capillary loops. There were multiple areas where the epithelial cell cytoplasm rested directly on the basement membrane with obliteration of the foot processes (Fig. 4). The main cell body of the podocyte and its cytoplasmic extensions were markedly flattened, and in areas the thickness of the podocyte cell cytoplasm was more or less equal to the thickness of the basement membrane (Figs. 4 and 5). In focal areas, the podocytes showed villous formations (Fig. 4). These villi were more numerous than in the control kidney, and corresponded with the increased number seen on scanning electron microscopy. No abnormalities were noted in the capillary endothelium, basement membrane, or mesangium.

Scanning electron microscopy. The normal structure of the glomerular capillary wall is shown in Fig. 6. From the main body of the podocyte, a large number

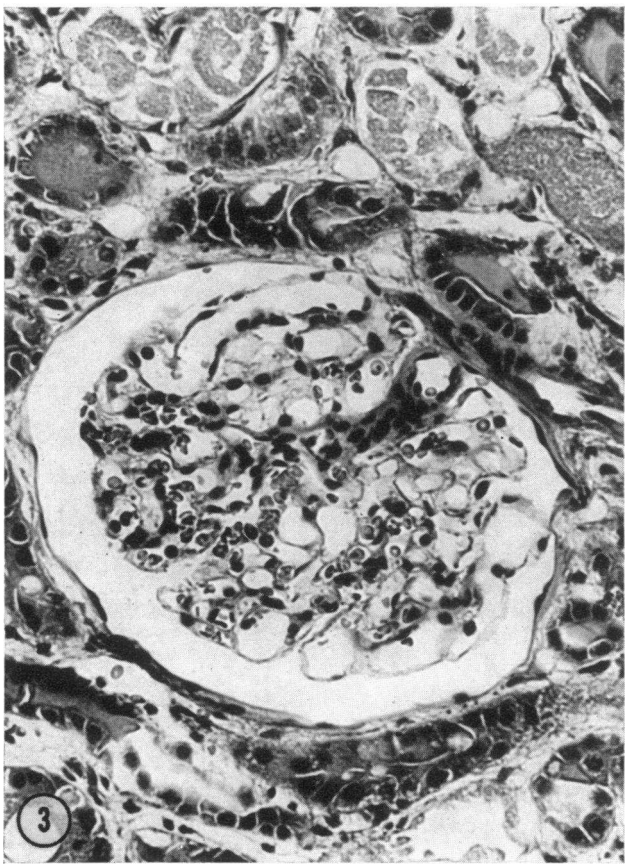


FIGURE 3 Histology from the experimental kidney. The photomicrograph shows patchy necrosis of the tubules. The glomerular capillary loops are dilated and contain erythrocytes. (Hematoxylin and eosin × 170)

of primary processes extended toward the capillary wall. A short distance away from the origin of the primary processes, many secondary processes emerged at right angles. Large number of fingerlike projections (foot processes) were seen emerging from both sides of secondary processes and a few from the primary processes. These foot processes covered the capillary walls and interdigitated with the same kinds of processes coming off the primary and secondary processes of other cell bodies. A few small villous extensions were seen coming off the surface of the main cell body of the podocytes and some of its processes. These projections extended towards the urinary space and were not attached to the capillary wall.

A representative section of a glomerulus of the experimental kidney is shown in Fig. 7. The glomerulus presented a shaggy surface with loss of the organized structure of the podocytes. It was hard to distinguish the main cell bodies of the podocytes from their processes. In few places where the main cell bodies of the podocytes could be discerned, they were seen to be somewhat shrunken and wrinkled and were resting directly on the capillary walls with no intervening space. There was marked crowding of the primary, secondary, and foot processes of the podocytes. In most of the areas examined, it was difficult to distinguish them separately (Fig. 7B). In the normal glomerulus, very few villi were seen projecting from the cell surface of the podocytes of its processes. However, in the experimental kidney, the podocytes and its processes presented a marked increase in the number of villi. Most of these villous structures were small and club-shaped. In most of the areas examined these villous processes were crowded together, presenting aggregated masses (Fig. 7B).

DISCUSSION

In the present study, the unilateral infusion of norepinephrine was utilized to produce acute oliguric renal failure in the dog. It was consistently found that a prolonged intrarenal infusion of 0.75 μg/kg per min of norepinephrine produced a fall in renal blood flow 48 h after administration in association with the essential cessation of urine flow on the involved side, while on the contralateral side, blood flow significantly increased 32%. This model of unilateral acute renal failure in the dog is reproducible, allows for the contralateral kidney to serve as an internal control, and can be produced without the complicating biochemical alterations of uremia. Histologically, the lesion is characterized by widespread tubular necrosis. In addition, a glomerular abnormality was found, which will be subsequently discussed.

As is shown in Table I, 48 h after norepinephrine administration, blood flow fell 39%. However, as shown in Table II and Fig. 1, there was no significant alteration in the intrarenal distribution of blood flow. Since systemic blood pressure was unchanged from the values obtained in the control period, this indicates a marked increase in renal resistance. In previous studies from this laboratory, we have shown that the intrarenal administration of much smaller doses of norepinephrine (24), angiotensin (24), and sympathetic nerve stimulation (25) also markedly increased renal resistance, but did not alter cortical blood flow distribution. Thus, these findings confirm the previous observation that renal vasoconstriction uniformly decreases cortical blood flow.

In the contralateral uninvolved kidney at 48 h, renal blood flow significantly increased 32%. The mechanism of this rise in flow in the control kidney is not clear, but may be similar to the alterations in renal hemodynamics which occur after unilateral nephrectomy or other situations in which functional renal mass is decreased in the contralateral kidney (26, 27). Concomitant with the increase in renal blood flow in the control kidney, there was a redistribution of cortical blood flow to the inner cortex. This is not surprising, since renal

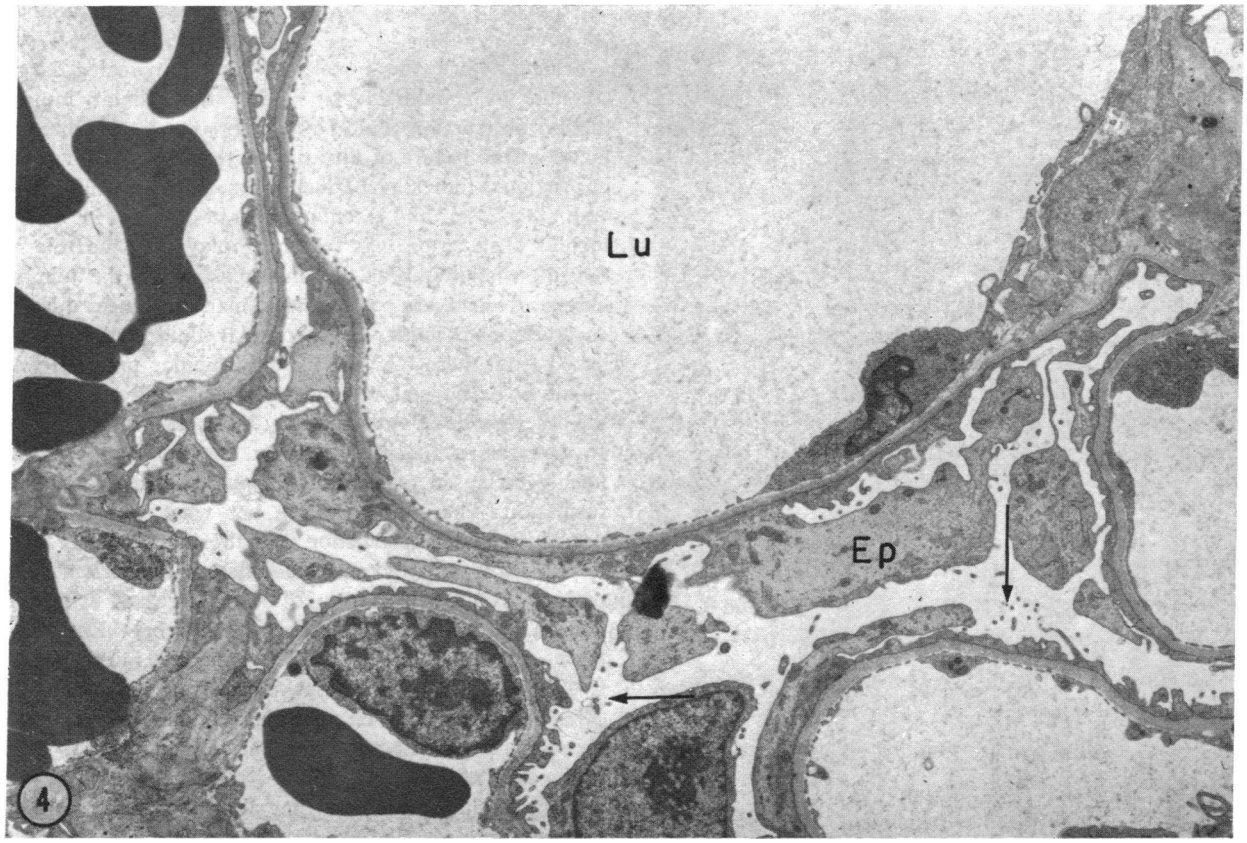


FIGURE 4 Transmission electron micrograph of glomerulus from experimental kidney. The capillary shows mild dilatation of lumen (Lu). Note extensive areas of fusion of foot processes. Notice also (at arrow) large number of villous structures close to the cell surface of the podocytes (Ep). Some of them can be seen to be continuous with the cytoplasm of the podocytes. (Uranyl acetate and lead citrate ×2,400).

vasodilation has been demonstrated to be associated with a decrease in the fractional distribution of blood flow to outer cortical nephrons (18, 28). In a recent study, McNay and Miyazaki found a similar change in regional flow distribution 8 days after unilateral nephrectomy (29). However, the interpretation of these data was complicated by the same directional changes in sham-operated control animals. In the present study, the norepinephrine kidney had no alteration in regional blood flow, while the contralateral vasodilated kidney demonstrated a consistent decrease in outer cortical nephrons.

After Ringer's loading, there was a 103% increase in renal blood flow in the experimental kidney in association with a redistribution of blood flow to inner cortical nephrons (Table I). Flow returned to the control levels in zones I and II and was increased above the levels in period I in the inner cortical zones. Additionally, blood flow further rose 26% in the control kidney, an increase primarily confined to inner cortical nephrons. These findings were quite similar to the changes noted by Blantz, Katz, Rector, and Selden (30) and Stein, Osgood, and Ferns (31) during volume expansion in normal dogs and presumably were also related to the intrarenal adjustments that occur during vasodilatation.

The reversal of the increase in renal resistance in the experimental kidney is consistent with a number of related observations. Ladefoged and Winkler (13) and Hollenberg and associates (32) have demonstrated that the administration of vasodilators can markedly increase renal blood flow in patients with acute renal failure. Jaenike found in rats with acute renal failure that saline loading could increase renal blood flow to normal levels with only a minimal improvement in total glomerular filtration rate (14). In addition, Fung and Thomson, using the same model as in the present study, have found that acetylcholine will reverse the renal vasoconstriction in the norepinephrine-infused kidney, although the dose-response curve is shifted to the right

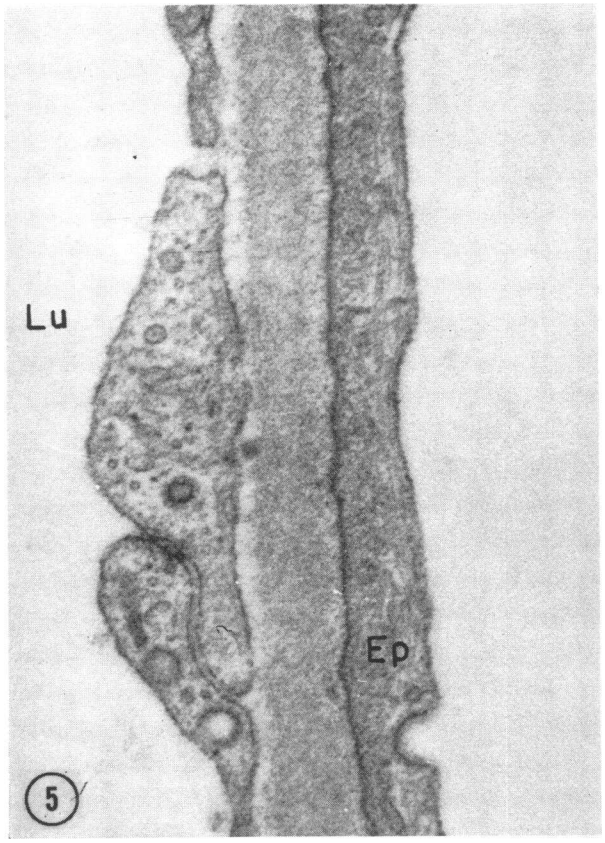


FIGURE 5 Transmission electron micrograph of glomerulus from experimental kidney shows marked flattening of the cell surface of podocyte (Ep) with obliteration of foot processes. (Uranyl acetate and lead citrate × 15,000).

(33). Thus, although the mechanism responsible for the continued renal vasoconstriction in the various models of acute renal failure is not known, it is clear that it can be reversed.

In spite of the increase in total blood flow in the experimental kidney during volume expansion to a level higher than in the control period, there was essentially no urine flow. Therefore renal vasoconstriction does not account for the oliguria in the norepinephrine model after saline loading. There are several possible explanations for this finding. First, reabsorption of filtrate across damaged tubular epithelia must be considered. However, it was found that the tubules were collapsed, no tubular fluid could be obtained, and lissamine green was visible only in the capillary circulation. Since it seems quite unlikely that a substantial amount of fluid could be absorbed in the initial 10% of the proximal tubule not accessible to visualization, these findings would seem to indicate that filtrate formation had been essentially abolished in superficial nephrons. Although back leak of filtrate from deep nephrons cannot be excluded, it should be noted that the histologic changes found were uniform throughout the cortex.

Second, since the histologic studies revealed large amounts of cellular debris within the tubular lumen, tubular obstruction could be considered as a contributory factor in the failure of filtration in this model. However, in previous studies by Flamenbaum, McDonald, DiBona, and Oken (4) and Jaenike (3) in models in which tubular obstruction was felt to be playing some role in the oliguria, fluid was demonstrable within the tubular lumina, which were patent and in many instances dilated. In addition, in both studies tubular fluid could be collected from these tubules, and intratubular pressure was found to be normal in one study (4), and elevated in the other group of experiments (3) in obstructed tubules. In contrast, in the present study, the tubules were uniformly collapsed and there was no evidence of filtrate formation. These findings are not what would be expected if tubular obstruction was altering filtration.

Third, shunting of renal blood flow via an aglomerular pathway could explain the failure of increased urine formation in the presence of increased renal blood flow. However, in extraction studies using the microspheres, 99% of the 15-μm spheres were trapped within the glomerular capillary circulation, a finding which would be strongly against any quantitatively substantial shunt pathway.

Fourth, the parameters that alter the effective filtration pressure must be examined. The rate of filtration of a given nephron is equal to K_F ($\bar{P}_{GC} - P_T - \bar{\pi}_{GC}$), where K_F is the ultrafiltration coefficient, $\bar{P}_{GC}$ is the mean glomerular capillary hydrostatic pressure, P_T is the proximal tubular pressure, and $\bar{\pi}_{GC}$ is the mean glomerular capillary oncotic pressure. In an extensive set of ingenious studies, Brenner, Troy, Daugherty, Deen, and Robertson have directly measured glomerular dynamics in a breed of Wistar rats with surface glomeruli (34–37). These studies have shown that $\bar{P}_{GC}$ and the net glomerular ultrafiltration pressure (P_{UF}) are much lower than previously suggested. $\bar{P}_{GC}$ was found to be approximately 45 mm Hg and P_{UF} at the afferent arteriolar end of the glomerulus was 14 mm Hg (34). Since the glomerulus of the dog kidney is not accessible to micropuncture, the direct measurement of $\bar{P}_{GC}$ has not been obtained. However, using the stop flow pressure method, Israelit, Rector, and Seldin found an estimated $\bar{P}_{GC}$ of 61 mm Hg in hydropenic and saline-loaded dogs (38).

In the present studies, during saline loading, the plasma protein concentration fell to 4.1 g/100 ml, an oncotic pressure of 13 mm Hg. Since there was no filtration, this value will approximate $\bar{\pi}_{GC}$ while P_T was zero. In other words, the force opposing filtration was approximately 13 mm Hg. Thus, unless $\bar{P}_{GC}$ or K_F were markedly reduced, filtration should have occurred. From

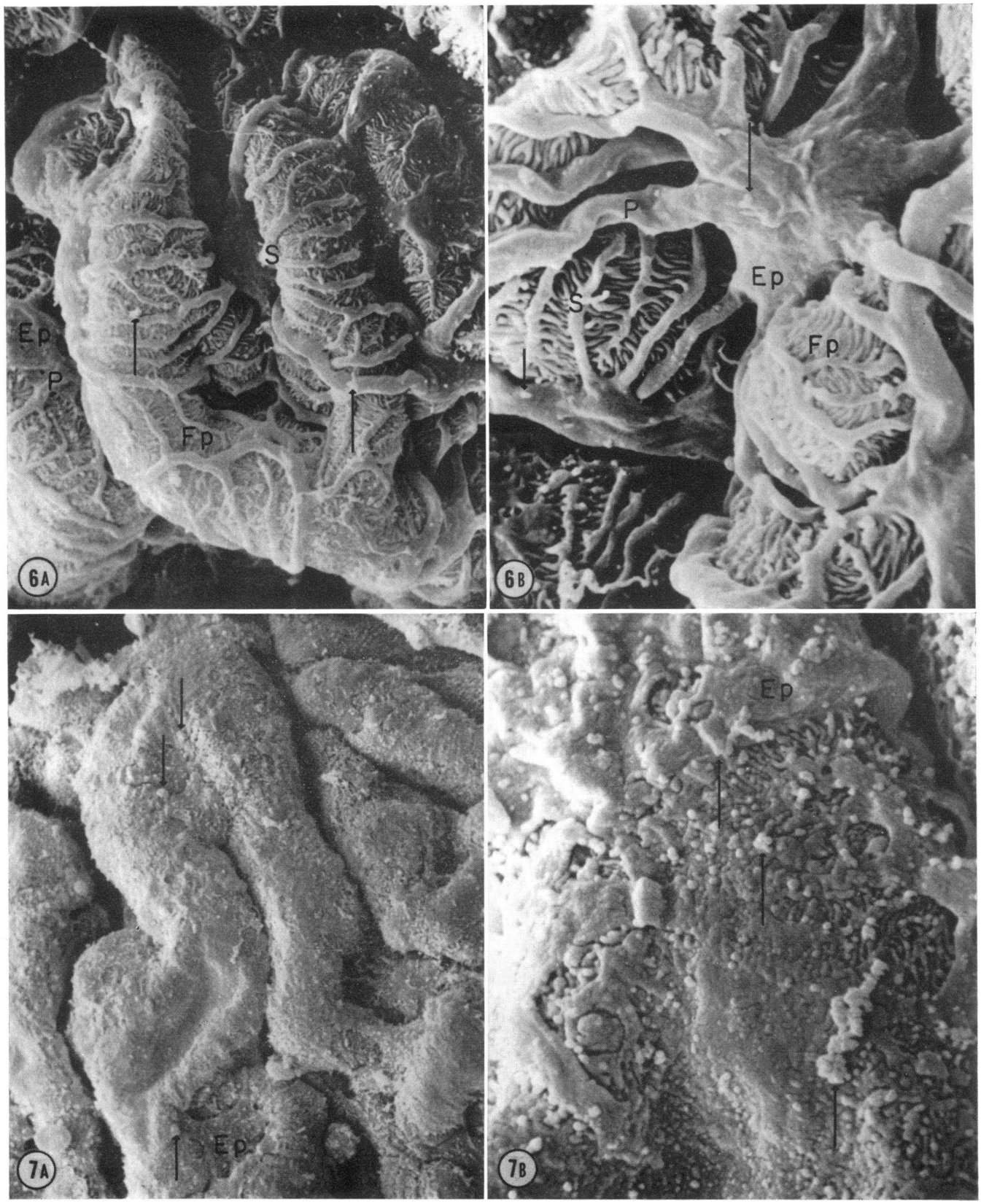
the changes in renal resistance and total blood flow after saline loading, it might be assumed that $\bar{P}_{GC}$ should have been normal or even somewhat increased. This would be the case unless there was a disproportionate fall in efferent arteriolar resistance. Even if we use the lower value of P_{GC} of 45 mm Hg and the relative afferent and efferent resistances directly found by Brenner and associates (35), afferent arteriolar resistance would have to increase 18% and efferent resistance would have to fall 76% to account for the magnitude of the increase in total blood flow without filtrate formation after volume expansion. This seems quite unlikely, since these same groups of investigators have noted a parallel fall in afferent and efferent resistance after volume expansion in normal rats (35) and in animals with renal ischemia (39).

Thus, there is no reason to expect that $\bar{P}_{GC}$ would be reduced to a level of 13 mm Hg, the force opposing filtration. From this analysis, it would seem possible that the failure of filtrate formation in this model was due to a marked fall in K_F. K_F is equal to a permeability factor, the hydraulic conductivity, times the surface area of the glomerulus. Since blood flow per nephron was normal or increased and all of the capillary loops appeared widely patent during saline loading (Fig. 3), it seems highly unlikely that effective surface area was decreased in this study. Because of the possibility that some alteration in capillary permeability could be involved in this process, transmission and scanning electron microscopy were performed. As is shown in Figs. 4–7, an unequivocal alteration in the epithelial structure of the glomerulus was noted. On transmission electron microscopy, loss of the normal foot process structure was found (Figs. 4 and 5). This was even more dramatically demonstrated in the scanning studies. There was marked disruption of the normal podocyte architecture (Fig. 7). the cell bodies were indistinct and wrinkled, and it was not possible to differentiate the primary and secondary processes as well as the terminal foot processes.

Hornych, Beaufils, and Richet have recently noted an abnormality in glomerular morphology by scanning microscopy after a 30–60-min intravenous infusion of angiotensin II in rats (40). The alterations noted were quite different from those of the present study. The primary changes were marked constriction of the capillary loops, swelling of the cell body and various processes, and fusion of the terminal foot processes. However, the basic podocyte structure was still readily identifiable. In addition, the juxtamedullary nephrons were totally spared. In the present study, the capillary loops were widely patent, the normal epithelial architecture was markedly altered, and the lesion involved all groups of cortical nephrons. In addition, the epithelial changes after angiotensin also occurred after a nonpressor dose and were invariably associated with proteinuria. Since these changes are quite like those reported in lipoid nephrosis (41), it is possible that this may be related to the proteinuria seen after the administration of renin (42, 43) or angiotensin II (40). The infusion of norepinephrine has not been reported to cause proteinuria.

Although the pathological changes found in the present study may have had no effect on glomerular dynamics, it seems more than coincidental that an alteration in glomerular capillary permeability is the most likely explanation for the physiologic findings in this study. It has been assumed that foot process fusion is associated with a generalized increase in glomerular capillary permeability. However, recent preliminary studies by Robson, Giangiacomo, Naqvi, Kienstra, and Ingelfinger are not compatible with this view (44). These investigators found in children with lipoid nephrosis a marked decrease in the clearance of polyvinylpyrrolidone molecules with a radius less than 15-Å, although the clearance of 45-Å molecules was increased. Thus, structural alterations of this type can in some manner impair the filtration of smaller molecules. Additionally, it should be noted that the lesions found in the norepinephrine model are much severer than in lipoid nephrosis. This was best demonstrated on scanning electron microscopy. In lipoid nephrosis, there is edema of all of the components of the epithelial structures but the basic architecture is still distinct (41). In contrast, as is demonstrated in Fig. 7, the epithelial surface was shaggy and it was difficult to discern the organized structure of the podocyte. In addition, it was not possible to differentiate the primary, secondary, and foot processes of the podocytes readily. This was due, in part, to the large number of aggregated villi. It is possible that this severer epithelial abnormality could even impede filtrate movement across the glomerular capillary wall.

As in previous studies of glomerular morphology in acute renal failure (45), little abnormality was noted by light microscopy. To our knowledge, there have been no studies utilizing scanning techniques and only a very limited number in which transmission electron microscopy has been used to evaluate acute renal failure. Dalgaard and Pedersen studied five cases of varying types of acute renal failure and found no abnormality (46). Similarly, Dalgaard found no glomerular abnormality in three patients with prolonged hypotension (47). In contrast, Suzuki and Mostofi noted swelling of the epithelial and mesangial cells in a period between 30 min and 16 h after glycerin injection in rats (48). These lesions tended to regress 24 h after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injection. Thus there are really few data available to determine whether or not this type of lesion is indeed present in other types of acute renal failure.

In summary, these studies suggest that an alteration in glomerular permeability may be responsible, at least in part, for the failure of filtrate formation in a model of unilateral acute renal failure in the dog. Although this is not meant to imply that this abnormality is operative in other forms of acute renal failure, these findings demonstrate the need for further evaluation of the possibility.

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FIGURE 6 Scanning electron micrograph from control kidney.
A. The capillary walls are seen to be surrounded by primary, secondary, (s) and foot processes (Fp) of the podocytes. These processes are separated from each other by a small amount of space. Notice few villi emerging from the cell surface and its processes (arrow) (× 1,000).
B. This photograph shows the main cell body of podocyte (Ep) with large number of primary (p) and secondary processes (s). The foot processes (Fp) are mainly seen to emerge from both sides of the secondary processes. Very few villi (arrow) are seen to emerge from the cell surface (× 5,000).
FIGURE 7 A. Scanning electron micrograph of glomerulus from experimental kidney. The glomerular loops present a rough and uneven surface. The podocyte (Ep) and its processes are in general indistinguishable. Notice large numbers of villi (arrow) emerging from the podocyte (× 1,000).
B. The podocyte and its processes are crowded together with loss of space in between the processes. Also notice the wrinkling of the cell surface. Large number of aggregated villi are seen emerging from the surface of cell and its processes (arrow) (× 5,000).

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