JOURNAL OF SURGICAL RESEARCH 26, 285–292 (1979)

The Effect of Bacterial Pyrogen on the Distribution of Intrarenal Blood Flow

JACK L. CRONENWETT, M.D., AND S. MARTIN LINDENAUER, M.D.[1]

Department of Surgery, Section of General Surgery, University of Michigan Medical Center, Ann Arbor, Michigan 48104, and Vascular Research Laboratory, Veterans Administration Medical Center, Ann Arbor, Michigan 48105

Presented at the Annual Meeting of the Association for Academic Surgery, Cleveland, Ohio, November 12–15, 1978

Hemodynamic and biochemical measurements were made before and 90 min after 50 μg of *Pseudomonas*-derived pyrogen was infused into nine awake dogs. Six control dogs received no pyrogen. Total renal blood flow (RBF) and intrarenal distribution of blood flow to four equal cortical zones (outer to juxtamedullary) were determined by the radioactive microsphere method. Pyrogen administration produced tachycardia, pyrexia, polyuria, natriuresis, and increased RBF. Cardiac output, arterial pressure, hematocrit, creatinine clearance, and white blood cell count did not change significantly. Despite increased total RBF, distribution of blood flow within the renal cortex did not change significantly after pyrogen administration. When compared with the effect of live bacteria, these studies suggest that polyuria and natriuresis result from any increased juxtamedullary blood flow without being dependent on redistribution, per se. Furthermore, the detrimental renal effect of bacterial sepsis may be related to the relative outer cortical hypoperfusion in contrast to the benign renal effect of pyrogen where no redistribution of blood flow occurs.

INTRODUCTION

The effects of bacterial pyrogen upon the kidney have been known since Homer Smith's observations in 1938 [5]. The increased renal blood flow (RBF), diuresis, natriuresis, and unchanged glomerular filtration rate subsequently have been well documented [3, 6, 7, 11].

Clinical studies of patients in septic shock have documented similar findings of renal hyperemia, inappropriate polyuria, and natriuresis [15], suggesting the possibility that much of the renal response during sepsis could be related to a bacterial pyrogen effect. However, in contrast to the benign effect of the pyrogen alone, renal failure is often associated with bacterial sepsis. A comparison of these effects upon the kidney, therefore, might elucidate the etiologic role of bacteria, per se, in the detrimental effects of sepsis upon the kidney.

Using microsphere techniques in awake dogs, the renal response to live bacterial sepsis has previously been reported from this laboratory [9]. The most striking finding was a redistribution of intrarenal blood flow away from the outer cortex to the juxtamedullary cortex, and by inference, the medulla. Such a redistribution has been described in most other experimental settings in which renal vasodilation occurs [14]. The effect of pyrogen administration on the distribution of intrarenal blood flow, however, has not been previously reported.

This study was designed to measure in awake dogs the effect of pyrogen upon the intrarenal blood flow distribution using microsphere techniques. These data, when combined with previous studies from this laboratory [9], allow a comparison between the renal effect of live bacterial sepsis and pyrogen alone.

[1] To whom requests for reprints should be addressed: B4962 Clinical Faculty Office Building, The University of Michigan Medical Center, Ann Arbor, Mich. 48109.

0022-4804/79/030285-08$01.00/0

METHODS

Fourteen female dogs (14–23 kg; mean 19 ± 5 kg) were allowed free access to food and water until they were briefly anesthetized with intravenous thiamylal sodium (10 mg/kg). During this light anesthesia, a transfemoral 7F side-hole cardiac catheter was positioned in the left ventricle. A transfemoral 18.5-gauge polyethylene catheter was placed in the abdominal aorta near the level of the renal arteries. A similar catheter was placed in the brachial artery for pressure recording. A Swan–Ganz thermodilution catheter was inserted into the pulmonary artery via the external jugular vein and a Foley catheter was inserted into the urinary bladder. The incisions for catheter placement were closed and the dogs allowed to recover from the anesthesia. When the animals were completely awake, they were placed in an upright Pavlov stand for the remainder of the experiment. Mean arterial pressure and rectal temperature were recorded continuously on a Hewlett–Packard Series 8800 recorder. Cardiac output was measured intermittently by thermodilution using an Edwards Model 9500 computer. Urine was collected at 15-min intervals and analyzed for creatinine and sodium concentration. Simultaneous serum samples for sodium, creatinine, white blood cell count, and hematocrit were obtained. After the animals were stable in the Pavlov stand, baseline data were collected for 60 min. At the end of this sampling period, intrarenal blood flow distribution and total RBF were measured using a microsphere technique. Carbonized microspheres labeled with ^{141}Ce (15 ± 5 μm diameter in 20% dextran) were used (Nuclear Products, 3M Co.). Microspheres (400,000) were injected through the left ventricular catheter and flushed with 20 ml of heparinized saline over 10 sec. A blood reference sample was withdrawn simultaneously from the abdominal aortic catheter using a Harvard withdrawal pump at the rate of 20 ml/min for 1 min beginning 5 sec before microsphere injection.

Following this determination, six control dogs received only sterile D_5W, while eight experimental dogs received 50 μg *Pseudomonas*-derived pyrogen (Piromen).[2] Data were collected for 90 min as described above. Intrarenal blood flow distribution then was measured using ^{85}Sr-labeled microspheres. Approximately 1 hr later, the animals were sacrificed and the kidneys removed and fixed in 10% formalin solution. During the entire experiment, fluid administration was restricted to 0.5 ml/min D_5W.

Total RBF was calculated by the reference sample technique [1, 8]. Both entire kidneys from each animal plus the aortic reference blood samples were counted using a sodium iodide crystal scintillation detector. Samples were placed into plastic containers and rotated to eliminate geometric variance. The details of the calculations have been previously described [8, 15].

Following isotope counting of the entire kidney, each kidney was divided in half coronally. Three sections of renal cortex were taken from each half kidney and divided into four equal layers by depth (from outer to inner cortex, labeled Layers 1 through 4). The six samples of each cortical layer were then pooled, weighed, and counted for both isotopes using a lithium-drifted germanium detector. Computer analysis [8] resolved microsphere distribution into the percentage distribution of blood flow to each of the four cortical layers for each isotope. Using an approximation for renal ellipsoidal geometry, the percentage distribution was calculated to each of four equal-volume cortical zones, labeled Zones 1 through 4 from the outer to the inner cortex [8, 16]. Zonal perfusion rate, or absolute blood flow to each zone, was calculated [8]. The 30-min period prior to microsphere injection was selected for comparison of hemodynamic, serum, and urine variables.

[2] Piromen was supplied by Travenol Laboratories, Morton Grove, Ill. This material is a nonprotein complex polysaccharide derived from *Pseudomonas* organisms.

Kidney specimens were examined by light microscopy. Statistical significance was determined by Student's two-tail *t* test for paired data. Sterile technique was used throughout the experiment and blood cultures obtained from each animal at the conclusion of the procedure were sterile.

RESULTS

Cardiac index did not change significantly after injection of Piromen (pyrogen dogs) or D_5W (control dogs) (Table 1). Mean arterial pressure also did not change in either control or pyrogen animals (Table 1). Heart rate did not change significantly in control animals. Pyrogen animals, however, demonstrated a significant tachycardia after Piromen injection (Table 1). As expected, rectal temperature increased significantly after pyrogen administration (40.0 to 41.4°C), but was unchanged in control animals (Table 1). White blood cell count increased slightly during the experiment in control animals, while decreasing insignificantly in pyrogen dogs (Table 2). Hematocrit and creatinine clearance did not change significantly in either group (Table 2). Control animals had stable urine output and sodium excretion during the experiment (Table 2). Pyrogen animals, however, demonstrated both polyuria and natriuresis, with a fourfold increase in both urine output and sodium excretion after Piromen injection (Table 2). This was associated with a significant increase in RBF, while RBF in control animals did not change (Table 1).

Distribution of intrarenal blood flow was analyzed for both right and left kidneys in all animals. Since there was no significant difference, the mean of right and left kidney values was utilized. Uncorrected distribution of blood flow (Layers 1–4) and corrected distribution of blood flow (Zones 1–4) showed a gradual decrease in percentage distribution from the outer cortex (Zone 1) to the juxtamedullary cortex (Zone 4) (Table 3). Control animals had stable distribution of blood flow during the experiment, with Zones 1, 3, and 4 statistically equivalent after injection and only Zone 2 showing a slight decrease in percentage distribution (Table 3 and Fig. 1). Pyrogen animals also demonstrated unchanged dis-

TABLE 1

PHYSICAL PARAMETERS BEFORE AND AFTER PYROGEN TREATMENT

		Baseline	After injection	*P* value
Cardiac index (liters/min/m²)	Control	3.6 ± 0.2[a]	3.3 ± 0.3	NS
	Pyrogen	3.6 ± 0.2	3.3 ± 0.3	NS
Mean arterial pressure (mm Hg)	Control	140 ± 5	145 ± 5	NS
	Pyrogen	143 ± 4	142 ± 7	NS
Heart rate (bpm)	Control	143 ± 11	135 ± 11	NS
	Pyrogen	132 ± 5	146 ± 8	<0.006
Rectal temperature (°C)	Control	40.3 ± 0.2	40.3 ± 0.2	NS
	Pyrogen	40.0 ± 0.1	41.4 ± 0.1	<0.0001
Renal blood flow[b] (ml/min)	Control	226 ± 20	220 ± 25	NS
	Pyrogen	230 ± 18	286 ± 24	<0.004
Renal blood flow per gram (ml/min/g)	Control	5.2 ± 0.3	5.0 ± 0.4	NS
	Pyrogen	4.9 ± 0.4	6.1 ± 0.4	<0.0008

[a] Mean ± SEM.

[b] Renal blood flow is expressed as the mean value of right and left kidneys.

TABLE 2

ANALYSIS OF URINE AND BLOOD SAMPLES TAKEN BEFORE AND AFTER PYROGEN TREATMENT

		Baseline	After injection	*P* value
White blood cell count (cells/mm^3)	Control	13,000 ± 2,600[a]	16,600 ± 3,500	<0.03
	Pyrogen	11,200 ± 850	6,100 ± 1,800	NS
Hematocrit (vol%)	Control	42.8 ± 2.6	39.2 ± 2.6	NS
	Pyrogen	40.4 ± 2.3	42.6 ± 1.8	NS
Urine output (ml/min)[b]	Control	0.34 ± 0.18	0.39 ± 0.22	NS
	Pyrogen	0.18 ± 0.02	0.80 ± 0.22	<0.02
Creatinine clearance (ml/min)[b]	Control	73 ± 14	83 ± 6	NS
	Pyrogen	86 ± 9	102 ± 18	NS
Sodium excretion (μeq/min)[b]	Control	13 ± 6	25 ± 11	NS
	Pyrogen	25 ± 13	100 ± 29	<0.03

[a] Mean ± SEM.

[b] Expressed as the sum of both kidneys, i.e., total animal values.

tribution of RBF after injection, despite the significant increase in total RBF (Table 3 and Fig. 2). Absolute zonal blood flow, or perfusion, did not change significantly in control animals (Table 4 and Fig. 3). In pyrogen animals, blood flow increased in each zone after Piromen injection, although this achieved statistical significance only in Zone 3 (Table 4 and Fig. 4).

Microscopic examination of renal tissue

TABLE 3

PERCENTAGE DISTRIBUTION OF INTRARENAL BLOOD FLOW

		Baseline	After injection	*P* value
Layer 1	Control	28.8 ± 1.6[a]	30.0 ± 2.5	NS
	Pyrogen	32.0 ± 2.2	32.6 ± 1.4	NS
Layer 2	Control	31.7 ± 1.1	28.0 ± 1.3	<0.04
	Pyrogen	28.5 ± 0.7	25.8 ± 1.0	<0.03
Layer 3	Control	23.8 ± 0.7	22.8 ± 1.3	NS
	Pyrogen	22.6 ± 0.7	22.3 ± 0.8	NS
Layer 4	Control	15.2 ± 1.2	19.3 ± 2.5	NS
	Pyrogen	17.3 ± 1.9	19.6 ± 1.1	NS
Zone 1	Control	37.3 ± 1.6	39.1 ± 2.6	NS
	Pyrogen	41.1 ± 2.4	43.1 ± 1.4	NS
Zone 2	Control	34.0 ± 0.9	29.8 ± 1.0	<0.004
	Pyrogen	30.0 ± 0.8	27.8 ± 1.2	NS
Zone 3	Control	19.7 ± 0.6	19.2 ± 1.3	NS
	Pyrogen	18.5 ± 0.8	18.1 ± 0.6	NS
Zone 4	Control	9.0 ± 0.8	11.5 ± 1.8	NS
	Pyrogen	10.3 ± 1.3	11.5 ± 0.7	NS

[a] Mean ± SEM.

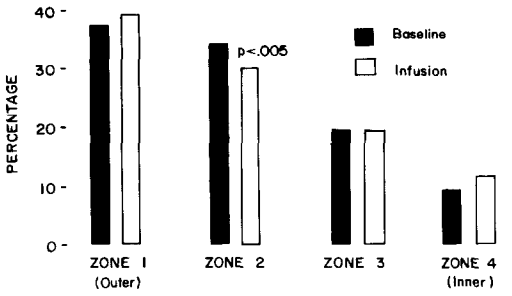

FIG. 1. Percentage distribution of blood flow to four cortical zones in control animals during baseline and after sterile D_5W injection.

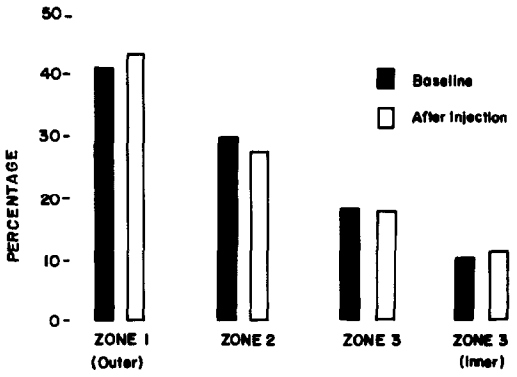

FIG. 2. Percentage distribution of blood flow to four cortical zones in pyrogen animals during baseline and 90 min after injection of pyrogen.

revealed that microspheres lodged in distal capillary loops of glomeruli. No microspheres were seen in the medulla, and no clumping of spheres was observed. Control animals had histologically normal kidneys. Pyrogen animals showed polymorphonuclear leukocytes within the capillary loops, but no other distinctive changes.

DISCUSSION

The pyrogen source used in this experiment was a commercially prepared polysaccharide complex derived from *Pseudomonas* organisms and previously used by other investigators [4, 11]. A Piromen dose was selected which produced a uniform febrile response and renal hyperemia during pilot experiments. The time for RBF distribution determination was at or near the peak of the flush phase reaction [4, 11]. The awake animal preparation employed in this study demonstrated stable hemodynamic and renal function over the course of the experiment and eliminated any effect of anesthesia.

The response to pyrogen administration has been well defined in both laboratory animals and humans. After a brief vasoconstrictive (chill) phase, there is an increase in renal plasma flow [3, 5, 11]. This is usually associated with increased cardiac output, but with a much greater portion of cardiac output perfusing the kidney, implying a selective renal vasodilator effect [3, 12].

TABLE 4

DISTRIBUTION OF ZONAL PERFUSION[a]

		Baseline	After injection	*P* value
Zone 1 perfusion	Control	7.1 ± 0.6[b]	7.1 ± 0.6	NS
	Pyrogen	7.4 ± 0.6	9.5 ± 0.6	NS
Zone 2 perfusion	Control	8.0 ± 0.6	6.8 ± 0.6	NS
	Pyrogen	6.7 ± 0.6	7.6 ± 0.6	NS
Zone 3 perfusion	Control	5.9 ± 0.4	5.7 ± 0.8	NS
	Pyrogen	5.4 ± 0.5	6.7 ± 0.5	<0.002
Zone 4 perfusion	Control	3.8 ± 0.4	4.9 ± 0.9	NS
	Pyrogen	4.2 ± 0.7	5.7 ± 0.4	NS

[a] Measured throughout in ml/min/g.
[b] Mean ± SEM.

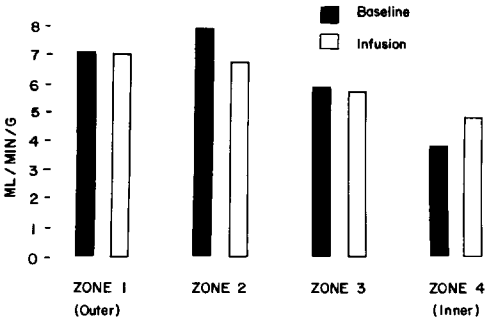

FIG. 3. Absolute blood flow (zonal perfusion) to each of four equal-volume cortical zones in control animals at baseline and after sterile D_5W injection.

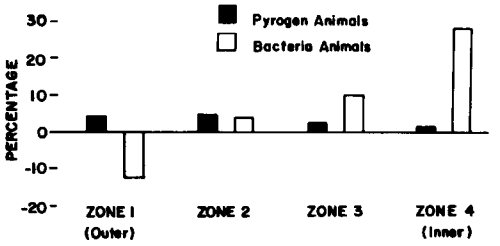

FIG. 5. Comparison of the percentage of blood flow distribution between dogs injected with pyrogen and a previous study where dogs received live bacteria. The change represents the difference from control animals in the same experimental model.

This renal effect is thought to be humoral, since denervated [13] or transplanted [6] kidneys exhibit the same response. It has not been possible in at least one experiment, however, to isolate an endogenous pyrogen [7]. Since the response is not blocked by antipyretics, increased RBF and cardiac output are not due to fever alone [3, 6, 11].

The present study confirmed the selective renal vasodilator effect of pyrogen and additionally demonstrated that increased RBF occurs without a change in intrarenal distribution. This is in contrast to most other experimental situations where vasodilation is associated with a shift of blood flow toward the medulla and away from the outer cortex (saline diuresis, hemorrhage, hypotension, bradykinin, acetylcholine, and prostaglandin E infusion) [14]. This differs from the response of dogs experiencing live bacterial sepsis in an otherwise identical experimental model [9]. This can be seen in Fig. 5 where the present data are compared with comparable data for live bacterial sepsis [9]. Pyrogen animals showed less than 5% change in distribution to all cortical zones when compared to control animals. Bacteria animals, however, demonstrated a dramatic shift of blood flow away from the outer cortical zones toward the inner, juxtamedullary cortex. Similarly, the data for absolute blood flow (zonal perfusion) are compared in Fig. 6 for pyrogen and bacteria dogs. Pyrogen animals showed uniformly a 30% flow increase to all four cortical zones corresponding to a 24% increase in total RBF. Bacteria animals, however, exhibited progressively larger increases in flow toward the inner cortex, with a 73% increased perfusion of the juxtamedullary cortex.

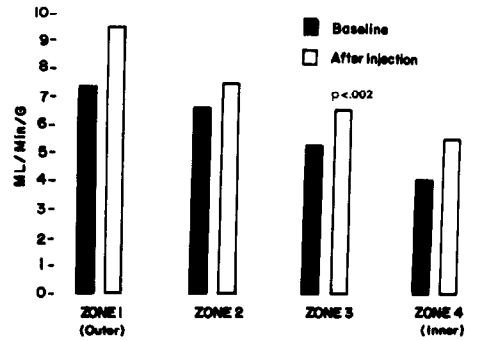

FIG. 4. Absolute blood flow (zonal perfusion) to each of four equal-volume cortical zones in pyrogen animals at baseline and after pyrogen injection.

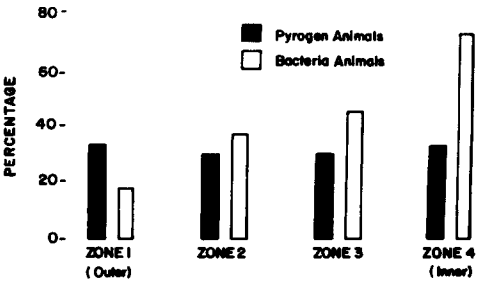

FIG. 6. Comparison of the change in zonal perfusion between dogs after pyrogen injection and dogs after live bacterial infusion. The change represents the difference from control animals in the same experimental model.

Despite these differences in flow distribution, both pyrogen and bacterial sepsis [9, 15] produced significant polyuria and natriuresis. In the present study, urine volume and sodium excretion both increased proportionately after pyrogen administration (400% increase) with urine concentration remaining the same. This can be explained by washout of the medullary concentration gradient by increased RBF [10, 14]. This decreases water reabsorption in Henle's loop and in collecting tubules, so that water diuresis results. Furthermore, decreased filtration fraction results in lower peritubular osmotic pressure, less proximal tubular sodium reabsorption and natriuresis [17]. Finally, decreased water reabsorption in the descending limb of Henle's loop leads to a decreased sodium concentration in the ascending loop and thus less sodium reabsorption, with natriuresis [10]. Brandt *et al.* reported that pyrogen abolished the renal response to antidiuretic hormone [4], which would be expected if the medullary concentration gradient were eliminated by increased medullary blood flow.

Since polyuria and natriuresis were noted in pyrogen dogs without redistribution of RBF, it can be argued that the redistribution of RBF reported in septic dogs [9] was not responsible for polyuria and natriuresis, but rather that increased total RBF alone is sufficient to explain this. However, polyuria and natriuresis also occurred in septic dogs with unchanged total RBF, but with redistributed and increased juxtamedullary flow. This suggests that any mechanism which increases juxtamedullary (and hence medullary) blood flow will decrease the medullary interstitial concentration gradient with resulting natriuresis and polyuria.

These data in pyrogen animals demonstrate that changes in distribution of RBF reported during bacterial sepsis cannot be explained on the basis of fever, vasodilation, or endogenous pyrogen alone, but rather are a specific bacterial effect. While pyrogen administration has no detrimental long-term effect on renal function, bacterial sepsis has often been associated with renal failure. The unchanged RBF distribution after pyrogen administration and the decreased outer cortical RBF noted during bacterial sepsis suggest that this redistribution may be detrimental to the kidney. Renal arteriograms in septic patients have shown that outer cortical blood flow was apparently reduced [2]. The significance of this redistribution with respect to development of renal failure in sepsis, however, awaits further laboratory and clinical investigation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Mr. Revius Williams, Mr. Larry Starks, and Mr. Larry Brown for technical assistance and animal care; Mr. John Jones, Phoenix Memorial Laboratory, The University of Michigan, for isotope analysis; and Dr. Theodore Beals, Veterans Administration Medical Center, Ann Arbor, for histologic analysis.

REFERENCES

1. Arruda, J. A. L., Boonjaren, S., Westenfelder, C., and Kurtzman, N. A. Measurement of renal blood flow with radioactive microspheres. *Proc. Soc. Exp. Biol. Med.* **146:** 263, 1974.
2. Arthachinta, S., Sitprija, V., and Kashemsant, U. Selective renal angiography in renal failure due to infection. *Aust. Radiol.* **18:** 446, 1974.
3. Bradley, S. E., Chasis, H., Goldring, W., and Smith, H. W. Hemodynamic alterations in normotensive and hypertensive subjects during the pyrogenic reaction. *J. Clin. Invest.* **24:** 749, 1945.
4. Brandt, J. L., Ruskin, H. D., Zumoff, B., Castleman, L., and Zuckerman, S. Inhibition of renal tubular responsiveness to antidiuretic hormone by pyrogens. *Proc. Soc. Exp. Biol. Med.* **88:** 451, 1955.
5. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W. The control of renal blood flow and glomerular filtration in normal man. *J. Clin. Invest.* **17:** 683, 1938.
6. Cooper, K. E., Cranston, W. I., Dempster, W. J., and Mottram, R. F. Pyrogen-induced vasodilatation in the transplanted kidney. *J. Physiol. (Lond.)* **155:** 21P, 1961.
7. Cranston, W. I., Vial, S. U., and Wheeler, H. O. The relationship between pyrogen-induced renal vasodilation and circulating pyrogenic substances. *Clin. Sci.* **18:** 579, 1959.
8. Cronenwett, J. L., and Lindenauer, S. M. Distribution of intrarenal blood flow following aortic

clamping and declamping. *J. Surg. Res.* **22:** 469, 1977.
9. Cronenwett, J. L., and Lindenauer, S. M. Distribution of intrarenal blood flow during bacterial sepsis. *J. Surg. Res.* **24:** 132, 1978.
10. Earley, L. E., and Friedler, R. M. The effects of combined renal vasodilatation and pressor agents on renal hemodynamics and the tubular reabsorption of sodium. *J. Clin. Invest.* **45:** 542, 1966.
11. Gombos, E. A., Lee, T. H., Solinas, J., and Mitronic, M. Renal response to pyrogen in normotensive and hypertensive man. *Circulation* **36:** 555, 1967.
12. Grimby, G. Renal clearance at rest and during physical exercise after injection of bacterial pyrogen. *J. Appl. Physiol.* **20:** 137, 1965.
13. Hiatt, E. P. The effect of denervation on the filtration rate and blood flow in dog kidneys rendered hyperemic by administration of pyrogen. *Amer. J. Physiol.* **136:** 38, 1942.
14. Lameire, N. H., Lifschitz, M. D., and Stein, J. H. Heterogeneity of nephron function. *Annu. Rev. Physiol.* **39:** 159, 1977.
15. Lucas, C. E., Rector, F. E., Werner, M., and Rosenberg, I. K. Altered renal homeostasis with acute sepsis. *Arch. Surg.* **106:** 444, 1973.
16. McNay, J. L., and Abe, Y. Redistribution of cortical blood flow during renal vasodilatation in dogs. *Circ. Res.* **27:** 571, 1970.
17. Stein, J. H., Ferris, T. F., Huprich, J. E., Smith, T. C., and Osgood, R. W. Effect of renal vasodilatation on the distribution of cortical flow in the kidney of the dog. *J. Clin. Invest.* **50:** 1429, 1971.