

## Superficial and Deep Juxtaglomerular Apparatus Renin Activity of the Rat Kidney EFFECT OF SURGICAL PREPARATION AND NaCl INTAKE

Walter Flamenbaum, Robert J. Hamburger

*J Clin Invest.* 1974;54(6):1373-1381. <https://doi.org/10.1172/JCI107884>.

### Research Article

The intrarenal gradient of renin activity was determined in rats by using superficial (S) and deep (D) cortical juxtaglomerular apparatuses (JGA's), identified and microdissected after silicone-rubber compound injection. Angiotensin generated from single JGA's using partially purified sheep renin substrate was quantified by rat bioassay. When, in rats on a normal NaCl diet, silicone-rubber was injected into a carotid artery, alone or with abdominal aorta catheterization, S:D renin activity ratios were  $1.18 \pm 0.08$  (SEM) and  $1.21 \pm 0.12$ , respectively. The S:D renin activity ratios obtained when silicone-rubber was injected into the abdominal aorta ( $2.52 \pm 0.09$ ) or a chronic carotid artery catheter ( $3.44 \pm 0.40$ ) were significantly higher ( $P < 0.001$ ). The lower S:D renin activity ratios after carotid artery manipulation were due to significantly higher D-JGA renin activities. This increased D-JGA renin activity and the lack of a renin gradient appear to be related to acute carotid artery manipulation.

Alterations in JGA renin activity were examined relative to NaCl intake. 2 wk after high-NaCl diet the absolute net renin activity decreased ( $P < 0.001$ ) more in S ( $5.84 \pm 0.11$  ng AI-JGA<sup>1</sup>·h<sup>-1</sup>) than D ( $1.73 \pm 0.06$  ng AI-JGA<sup>1</sup>·h<sup>-1</sup>) JGA's, and the intrarenal renin gradient was lost (S:D-JGA renin activity,  $1.00 \pm 0.07$ ), as compared to the regular NaCl diet. 2 wk of a low-NaCl diet resulted in a greater ( $P < 0.01$ ) increase in S ( $14.28 \pm 1.47$  ng AI-JGA<sup>1</sup>·h<sup>-1</sup>) than D ( $9.62 \pm 1.19$  ng [...])

Find the latest version:

<https://jci.me/107884/pdf>



# Superficial and Deep Juxtaglomerular Apparatus Renin Activity of the Rat Kidney

## EFFECT OF SURGICAL PREPARATION AND NaCl INTAKE

WALTER FLAMENBAUM and ROBERT J. HAMBURGER with the technical assistance of M. HUDDLESTON and L. TEMPLEMAN

*From the Department of Nephrology, Division of Medicine, Walter Reed Army Institute of Research, Washington, D. C. 20012*

**ABSTRACT** The intrarenal gradient of renin activity was determined in rats by using superficial (S) and deep (D) cortical juxtaglomerular apparatuses (JGA's), identified and microdissected after silicone-rubber compound injection. Angiotensin generated from single JGA's using partially purified sheep renin substrate was quantified by rat bioassay. When, in rats on a normal NaCl diet, silicone-rubber was injected into a carotid artery, alone or with abdominal aorta catheterization, S:D renin activity ratios were  $1.18 \pm 0.08$  (SEM) and  $1.21 \pm 0.12$ , respectively. The S:D renin activity ratios obtained when silicone-rubber was injected into the abdominal aorta ( $2.52 \pm 0.09$ ) or a chronic carotid artery catheter ( $3.44 \pm 0.40$ ) were significantly higher ( $P < 0.001$ ). The lower S:D renin activity ratios after carotid artery manipulation were due to significantly higher D-JGA renin activities. This increased D-JGA renin activity and the lack of a renin gradient appear to be related to acute carotid artery manipulation.

Alterations in JGA renin activity were examined relative to NaCl intake. 2 wk after high-NaCl diet the absolute net renin activity decreased ( $P < 0.001$ ) more in S ( $5.84 \pm 0.11$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>) than D ( $1.73 \pm 0.06$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>) JGA's, and the intrarenal renin gradient was lost (S:D-JGA renin activity,  $1.00 \pm 0.07$ ), as compared to the regular NaCl diet. 2 wk of a low-NaCl diet resulted in a greater ( $P < 0.01$ ) increase in S ( $14.28 \pm 1.47$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>) than D ( $9.62 \pm 1.19$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>) JGA renin activity and a renin gradient (S:D-JGA renin activity) of  $1.75 \pm 0.12$ . These results demonstrate that NaCl intake clearly

influences total JGA renin content and may also affect the relative intrarenal distribution of renin activity.

## INTRODUCTION

The studies of Granger, Dahlheim, and Thurau (1) using a sensitive method for determination of renin activity of single juxtaglomerular apparatuses (JGA's)<sup>1</sup> failed to demonstrate a superficial to juxtamedullary cortical renin gradient in the rat. In contrast, a corticomedullary intrarenal renin gradient has been directly or indirectly demonstrated in a variety of other species (2-5). A recent study by de Rouffignac, Bonvalet, and Menard (6), published while the current study was in progress, demonstrated a significant superficial to deep renin gradient in the rat, as did a previous indirect histochemical study (7). The major methodologic difference between direct studies of glomerular renin activity which demonstrated a renin activity gradient (3, 6) and studies of juxtaglomerular renin activity which failed to demonstrate a gradient (1, 8) was that in the latter the carotid artery or jugular vein was manipulated while in the former they were not. Brown, Davies, Lever, Parker, and Robertson directly visualized glomeruli in the rabbit cortex (3) and de Rouffignac et al. (6) calculated individual renin content per glomerulus by quantifying the renin content of cortical fragments and estimating the number of glomeruli per fragment; Dahlheim, Granger, and Thurau (8) injected Evans blue dye into the jugular vein, and Granger et al. (1) injected silicone-rubber into the carotid artery.

The present study was designed, therefore, to evaluate the influence of various methods of surgical prepa-

A preliminary report of this study was presented at the Eastern Section meeting of the American Federation of Clinical Research 1973. *Clin. Res.* 21: 991.

Received for publication 10 May 1974 and in revised form 12 August 1974.

<sup>1</sup>Abbreviations used in this paper: AI, angiotensin I; AII, angiotensin II; D, deep; JGA, juxtaglomerular apparatus; S, superficial.

ration on the renin activity of single S and D-JGA's. In view of the proposed role of intrarenal renin as an effector mechanism for interrelating changes in nephron function to alterations in sodium chloride homeostasis (9, 10) additional studies of the effect of high- and low-NaCl intake were also performed. The results of the present study indicate that the rat, like other species (2-5), has a demonstrable intrarenal renin gradient on a regular NaCl diet. Furthermore, the results suggest that this gradient is markedly influenced by carotid artery manipulation and variations in NaCl intake.

## METHODS

### General

Male Sprague-Dawley rats<sup>a</sup> weighing 275-350 g were used throughout the study. Anesthesia was induced with intraperitoneal pentobarbital (35 mg/kg body wt), and the animals were studied on a thermostatically regulated (37°C) animal board. The diets used in the study were as follows: (a) *regular NaCl*, consisting of standard rat chow (287 meq Na<sup>+</sup>/kg, 207 meq K<sup>+</sup>/kg, G. L. Baking Co., Frederick, Md.) and tap water; (b) *high-NaCl*, standard rat chow and 1% NaCl solution as the sole source of fluid intake; (c) *low-NaCl*, sodium-deficient rat diet (2.0 meq Na<sup>+</sup>/kg, 282 meq K<sup>+</sup>/kg, General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio) and deionized tap water. Statistical analyses were performed according to Snedecor and Cochran (11). Results are presented as the mean±SEM. Comparison of values within each group were obtained by paired analysis and comparisons of the different experimental maneuvers by group analysis.

### Surgical preparation and microdissection

To evaluate the effect of surgical preparation on renin activity the following groups of rats were prepared after anesthesia:

#### GROUP A ("ACUTE CAROTID")

One carotid artery was catheterized with PE60 (ID 0.03 in, OD 0.048 in) polyethylene tubing containing heparinized saline (100 U/ml), care being taken to avoid excessive manipulation or dissection of the cervical structures.

#### GROUP B ("ACUTE AORTA")

The lower abdominal aorta was catheterized just above the bifurcation with PE60 or PE90 (ID 0.034 in, OD 0.05 in) polyethylene tubing containing heparinized saline, care being taken to avoid excessive manipulation of the abdominal viscera.

#### GROUP C ("DOUBLE CATHETERS")

One carotid artery and the lower abdominal aorta were catheterized as noted above.

<sup>a</sup>In conducting the research described in this report the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences—National Research Council.

#### GROUP D ("CHRONIC CAROTID")

One carotid artery was catheterized with a short length of PE60 polyethylene tubing containing heparin solution (1,000 U/ml); the tubing was heat sealed, buried subcutaneously and the skin incision closed with wound clips; group D ("chronic carotid") was studied 4-6 days after implantation of the catheter.

Tracheostomies were not performed and the abdomen was opened in all groups at the time of study for the placement of loose ligatures around the renal pedicles. After completion of surgery in groups A-C and the time of study in group D, blood pressure was intermittently monitored (P23Gc pressure transducer, Statham Labs, Hato Rey, Puerto Rico) for 30 min; all rats with a mean pressure less than 110 mm Hg being excluded from further study.

30 min after initial vascular catheterization (groups A-C) or after establishing catheter patency (group D) 1.0-1.5 ml of a silicone rubber compound (2 parts MV117 to 1 part MV diluent, plus 0.1 part MV curing agent, Canton Bio-Medicals, Boulder, Colo.), was injected as a bolus. The injected silicone-rubber compound allowed identification and microdissection of a JGA. In group C ("double catheters") the silicone-rubber compound was injected into the abdominal aorta. The kidneys were rapidly excised and quick frozen in a dry ice-acetone mixture. The frozen specimens were stored at -10°C. Midcoronal slices were subsequently microdissected by hand on a cold plate (ambient temperature <4°C) by using fine needles and a stereo-microscope according to a modification of the technique described by Grange et al. (1). Prior freeze-drying of the renal tissue, as used by Granger et al. (1), was not required for either microdissection or measurement of renin activity. A JGA was considered complete and suitable for assay if the glomerulus, a short segment of the afferent arteriole, and the contiguous tubular tissue between afferent and efferent arterioles could be identified (1). Superficial (S) JGA's were identified as those lying just beneath the outer cortical avascular zone and in an area of high glomerular density. Deep (D) JGA's were identified as those situated just above the typically waxy medullary tissue in an area of low glomerular density. Granger et al. (1), in contrast, identified a JGA as deep (or juxtamedullary) by the origin of its afferent arteriole from an arcuate or interlobar artery. The order of JGA microdissection, or S or D, was alternated.

### Determination of renin activity

#### PREPARATION OF RENIN SUBSTRATE

Renin substrate was prepared according to the method of Skinner (12) from sheep plasma obtained 6 days after bilateral nephrectomy. Before use, the sheep renin substrate was adjusted to pH 6.0 with 0.1 N HCl. In order to evaluate remaining angiotensinase activity 100 ng of angiotensin II (AII) (Hypertensin, Ciba Pharmaceutical Company, Nutley, N. J.) was added to 5.0 ml of sheep renin substrate and incubated for 3 h at 37°C. The recovery of added angiotensin, determined in 12 samples by using the rat bioassay system (see below), was 89.9±1.3%. This value is not different from the mean value of 91.7±3.2% determined by bioassay of 12 unincubated 5.0-ml samples of saline solution (pH 6.0) containing 100 ng of AII, indicating the sheep renin substrate preparation was relatively angiotensinase free. Furthermore, generation of angiotensin was not detected by the rat bioassay when six aliquots (5.0 ml each) of sheep renin substrate were incubated at 37°C for 3 h, indicating the absence of endogenous renin. Sheep

renin substrate levels were determined by incubating (37°C) eight aliquots (0.9 ml) in the presence of excess rat renin (prepared as previously described, 13) at pH 6.0 for 30 and 60 min. The generated angiotensin was determined by using the rat bioassay (see below) and was the equivalent of  $1,155 \pm 28$  ng/ml of generateable AII after 60 min of incubation.

#### BIOASSAY OF RENIN

**Generation and assay of angiotensin.** The generation of angiotensin and the determination of renin activity was performed with a modification of the methods of Dahlheim et al. (8) and Granger et al. (1). A single JGA was placed in a siliconized polyethylene tube containing 0.2 ml of cold (0–4°C) sheep substrate, sonicated at 25 W for two 3-s intervals using a 3.0-mm probe tip. While in a cold bath (0–4°C) 5  $\mu$ l each of  $\text{NH}_4\text{EDTA}$  (20 mg/ml of distilled water), neomycin sulfate (20 mg/50 ml of pH 6.0 citrate buffer), and Trasylol (aprotinin, 10,000 KIE/ml, FBA Pharmaceuticals, Inc., New York) was added to the JGA-renin substrate mixture to inhibit angiotensinase and converting enzyme activities. In preliminary studies the addition of diisopropyl fluorophosphate did not alter AII recovery or the endogenous generation of angiotensin and was, therefore, not utilized in the bioassay system. Incubation was carried out at 37°C for 60–120 min, the reaction being stopped by rapid freezing in dry ice-acetone. Renin activity was determined by quantifying the angiotensin thus generated in nonvagotomized, pentolinium tartrate-treated rats with AII as the standard (1, 8). The pressor response to 25  $\mu$ l of test solution was compared to the standard response of 0.5–5.0 ng of AII. The results are expressed as the equivalent of AII generated per JGA per hour of incubation ( $\text{ng AII} \cdot \text{JGA}^{-1} \cdot \text{h}^{-1}$ ).

**Validity of renin bioassay.** Additional experiments designed to evaluate the validity of this renin bioassay system were performed. The generation of angiotensin from JGA's and renin extracted from whole rat kidney (as previously described, 13) were compared by determining the renin activity of 12 samples of pooled JGA's alone and after the addition of a known amount, 28.6 ng AI/0.01 ml, of renal

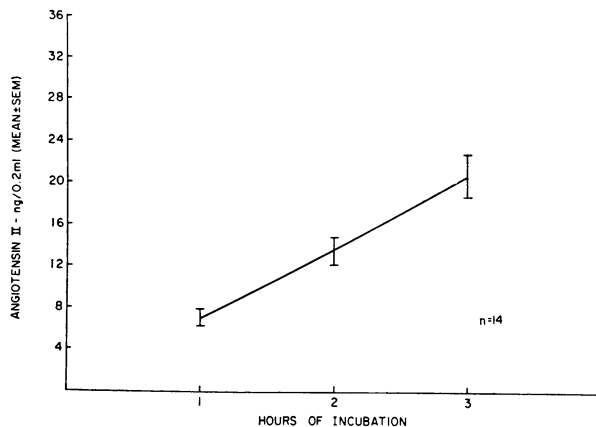


FIGURE 1 Generation of AII from 14 single JGA's after 1, 2, or 3 h of incubation at 37°C. The mean  $\pm$  SEM for total AII per 0.2 ml, the volume of sheep substrate utilized, is presented. See text for the mean values of AII generation per hour of incubation. Angiotensin generation was linear with time ( $r = 0.86$ ,  $P < 0.001$ ).

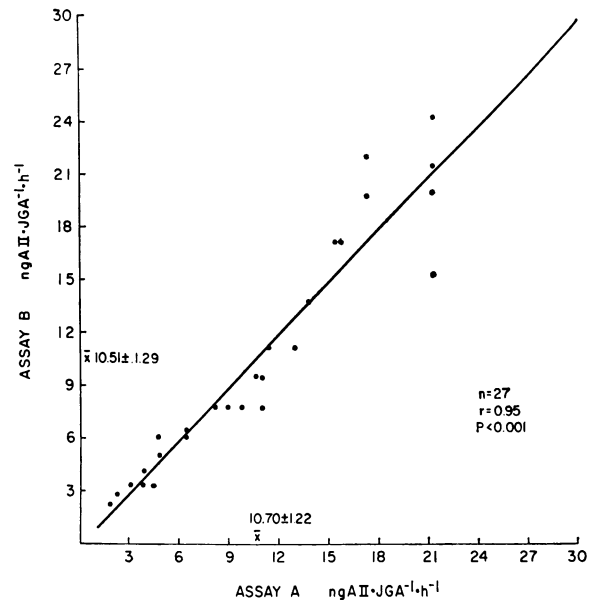


FIGURE 2 Comparison of duplicate bioassays (A and B) of AII generation from 27 single JGA's. A significant correlation ( $r = 0.95$ ,  $P < 0.001$ ) was observed between the two assays, and the mean ( $\bar{x}$ ) values for the comparative results are not significantly different.

renin. The observed angiotensin generation from JGA's + renal renin was  $93 \pm 3\%$  of the predicted value, suggesting that modifiers of the renin reaction were not present in either the renin assay method or the technique of JGA preparation. In order to insure that the angiotensin was generated from single JGA's in the presence of excess renin substrate 14 single S-JGA's were incubated for 1, 2, and 3 h and the generated angiotensin determined (Fig. 1). The mean values of  $7.03 \pm 0.88$ ,  $6.52 \pm 0.82$ , and  $6.90 \pm 0.68$   $\text{ng AII} \cdot \text{JGA}^{-1} \cdot \text{h}^{-1}$  for 1, 2, and 3 h are not statistically different, and a linear correlation of AII, in  $\text{ng}/0.2$  ml, with time was observed ( $r = 0.86$ ,  $P < 0.001$ ), indicating the presence of renin substrate in excess. The reproducibility of the bioassay was evaluated by replicate analysis of the angiotensin generated from a single JGA in six separate assay rats as well as by duplicate analysis of the angiotensin generated from 27 JGA's in different assay rats. The mean value for the replicate analyses of a single JGA in six assay rats was  $7.16 \pm 0.82$  (SD)  $\text{ng AII} \cdot \text{JGA}^{-1} \cdot \text{h}^{-1}$  and the results of duplicate analysis of 27 JGA's are depicted in Fig. 2. Taken together these results indicate that the bioassay is reproducible. The lower limit of sensitivity for the bioassay system was estimated from the determination of the AII content of serial dilutions, six each, of AII in normal saline (pH 6.0). The mean values obtained from samples containing 2.0, 1.0, 0.5, and 0.25  $\text{ng}/0.2$  ml were  $1.93 \pm 0.07$ ,  $0.96 \pm 0.08$ ,  $0.57 \pm 0.04$ , and  $0.09 \pm 0.04$   $\text{ng}/0.2$  ml, respectively. Since the coefficient of variance increased from a mean of 14.8% at the higher concentrations to 101% at a concentration of 0.25  $\text{ng}/0.2$  ml, the lower limit of sensitivity was taken as the generation of 0.5  $\text{ng AII} \cdot \text{JGA}^{-1} \cdot \text{h}^{-1}$ .

#### RADIOIMMUNOASSAY OF RENIN

To assess the comparability of angiotensin generated from single JGA's using the bioassay of AII and the radioim-

radioassay of angiotensin I (AI), 36 JGA's were evaluated with both techniques. Generated AI was quantified by a modification (13) of the radioimmunoassay procedure of Haber, Koerner, Page, Kliman, and Purnode (14) for AI. Standard AI, [<sup>125</sup>I]AI, and antiangiotensin antibody were purchased from New England Nuclear (Boston, Mass.). JGA's were obtained from animals on regular, high- and low-NaCl diets. For each diet six S- and six D-JGA's were prepared. As with the bioassay alone all incubations were performed at pH 6.0. Each JGA renin substrate mixture was divided into two aliquots before incubation: one aliquot was utilized for rat bioassay as noted above; the second aliquot was incubated for 30-60 min after the addition of 10 μl of 8-hydroxyquinoline (0.7 M) and 2.0 μl of BAL (20% solution in benzyl benzoate). 20-μl aliquots were utilized for radioimmunoassay. For the radioimmunoassay, renin activity of AI generated per JGA/h of incubation (ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>) represents the mean of a quadruplicate analysis of each specimen.

## RESULTS

*Effect of surgical preparation.* The results of renin activity determinations in the various groups (A-D) studied are presented in Table I, which contains the mean value (±SEM) for 4-9 JGA's in each animal as well as the overall mean±SEM and ranges for the S- and D-JGA's of each study group. In the acute carotid artery preparation (group A) the mean renin activity of S-JGA's in seven rats was 7.22±0.40 ng AII·JGA<sup>-1</sup>·h<sup>-1</sup>, not significantly different from the mean value of 6.27±0.13 ng AII·JGA<sup>-1</sup>·h<sup>-1</sup> in D-JGA's. In contrast, in six rats studied by using the acute abdominal aorta preparation (group B), the mean S-JGA value of 6.79±0.46 ng AII·JGA<sup>-1</sup>·h<sup>-1</sup> was significantly greater than the mean D-JGA value of 2.67±0.19 ng AII·JGA<sup>-1</sup>·h<sup>-1</sup> ( $P < 0.001$ ). While the mean S-JGA renin activities of groups A ("acute carotid") and B ("acute aorta") were not significantly different

( $P > 0.40$ ), the mean D-JGA renin activity in group B was significantly less than the value obtained in group A ( $P < 0.001$ ). Thus, while no cortical JGA renin gradient was observed with the acute carotid preparation, a significant gradient was observed with the acute abdominal aorta approach.

The influence of the site of vascular catheterization was further evaluated with group C ("double catheters") and group D ("chronic carotid") studies (Table I). In group C, in which the silicone-rubber compound was injected into the abdominal aorta, the mean S-JGA renin activity was not different from the mean D-JGA renin activity ( $P > 0.20$ ). Furthermore, the S- and D-JGA renin activities in group C (double catheters) were not significantly different ( $P > 0.10$ ) from the mean S- and D-JGA renin activities determined by using acute carotid artery catheterization alone (group A). These results suggest that it was not the infusion of silicone rubber compound per se which accounted for the lack of intrarenal cortical renin gradient with the acute carotid artery approach since the renin activities determined after the infusion of silicone rubber into the abdominal aorta of rats having a carotid artery catheter were not different from those determined after carotid artery catheterization alone. In contrast, in group D ("chronic carotid") the mean S-JGA renin activity was significantly greater than the mean D-JGA renin activity ( $P < 0.001$ ). In addition, mean S-JGA ( $P > 0.05$ ) and D-JGA ( $P > 0.40$ ) renin activities in group D ("chronic carotid") were not significantly different from the values observed in group B ("acute aorta"). These results suggest that the acute manipulation of the carotid artery was responsible for the loss of the cortical renin gradient observed after acute caro-

TABLE I  
S- and D-JGA Renin Activity in Rats on the Normal NaCl Diet. Effect of Method of Surgical Preparation

Group:	(A) Acute carotid artery preparation		(B) Acute abdominal aorta preparation		(C) Acute double catheters		(D) Chronic carotid artery preparation	
	S (38/7)*	D (38/7)	S (34/6)	D (45/6)	S (24/6)	D (24/6)	S (35/6)	D (35/6)
JGA renin activity:	ng AII·JGA <sup>-1</sup> ·h <sup>-1</sup>				ng AII·JGA <sup>-1</sup> ·h <sup>-1</sup>			
	7.27±0.68‡	6.05±0.85	7.46±1.76	2.88±1.02	6.72±0.99	5.94±1.09	6.26±0.54	2.27±0.31
	6.14±0.49	5.97±0.89	6.14±0.49	2.69±0.39	7.80±0.41	8.00±1.17	7.19±0.47	2.61±0.26
	6.17±0.82	5.11±0.95	6.00±0.68	2.37±0.36	8.31±0.61	6.94±0.71	8.21±0.71	1.59±0.36
	6.68±0.81	4.97±0.58	5.48±0.64	1.87±0.26	13.75±1.51	9.00±0.94	7.93±0.57	3.06±0.41
	8.47±0.71	6.01±1.18	7.15±0.54	3.09±0.31			9.87±0.62	2.62±0.39
	9.00±0.85	7.33±1.02	8.51±0.88	3.40±0.74			9.84±0.61	2.73±0.28
	6.81±0.60	8.44±0.63						
Mean±SEM for group	7.22±0.40	6.27±0.13	6.79±0.46	2.67±0.19	9.16±1.57	7.47±0.66	8.22±0.59	2.48±0.2
Range of values	3.53-11.16	2.17-11.14	3.75-15.00	0.83-7.12	4.48-19.89	3.22-12.94	5.47-12.16	0.95±4.93
P value	>0.10		<0.001		>0.20		<0.001	

\* The numbers in parentheses represent the number of JGA's studied over the number of animals studied.

‡ The mean±SEM are presented for each animal studied. Four to nine JGA's were assayed per animal.

TABLE II  
S and D-JGA Renin Activity in Rats on High- or Low-NaCl Diets

JGA renin activity:	High-NaCl diet		Regular NaCl diet		Low-NaCl diet	
	S (35/6)*	D (36/6)	S	D	S (35/6)	D (36/6)
	<i>ng AII·JGA<sup>-1</sup>·h<sup>-1</sup></i>					
	1.06±0.05‡	0.96±0.07			16.83±1.86	10.18±1.54
	1.30±0.07	1.13±0.12			22.43±0.93	16.83±1.53
	0.98±0.12	0.92±0.12			18.52±2.51	10.88±2.31
	0.58±0.06	0.80±0.10			19.28±1.37	10.88±1.26
	0.82±0.09	0.76±0.12			26.88±4.07	15.04±1.92
	0.96±0.07	1.08±0.14			22.50±1.91	9.92±2.13
Mean±SEM for group	0.95±0.10	0.94±0.06	6.79±0.46	2.67±0.19	21.07±1.47	12.29±1.19
Range of values	0.50-1.37	0.64-1.33			11.85-41.46	5.05-16.65
P value	>0.80		<0.001		<0.001	

\* The numbers in parentheses represent the number of JGA's studied over the number of animals studied.

‡ The mean±SEM are presented for each animal studied. Five to six JGA's were assayed per animal.

tid artery catheterization since a significant gradient was observed after chronic carotid artery catheterization.

*Effect of high- or low-NaCl diets.* Additional studies, utilizing only the acute abdominal aorta preparation, were performed 14 days after either the high- or low-NaCl diet. Results of these studies are contained in Table II and may be compared with group B ("acute aorta") studies obtained on a regular NaCl diet. The high-NaCl diet resulted in marked decreases in both mean S- and D-JGA renin activity to 0.95±0.10 and 0.94±0.06 ng AII·JGA<sup>-1</sup>·h<sup>-1</sup>, respectively, values not significantly different from each other ( $P > 0.80$ ) and significantly less than the values observed in rats on the regular NaCl diet ( $P < 0.001$ ). After the low-NaCl diet the mean renin activities in S-JGA's of 21.07±1.47 and D-JGA's of 12.29±1.19 ng AII·JGA<sup>-1</sup>·h<sup>-1</sup> were significantly different from each other ( $P < 0.001$ ) as well as significantly greater ( $P < 0.001$ ) than those observed on the regular NaCl diet.

*Radioimmunoassay vs. bioassay.* Fig. 3 depicts the renin activities determined by using the bioassay of generated AII and radioimmunoassay of AI from S- and D-JGA's obtained in rats on regular, high-, or low-NaCl diets. A linear relationship ( $r = 0.95$ ,  $P < 0.01$ ) was observed over a range of values from 0.86 to 21.7 ng AII·JGA<sup>-1</sup>·h<sup>-1</sup> (bioassay) and from 1.79 to 58.65 ng AI·JGA<sup>-1</sup>·h<sup>-1</sup> (radioimmunoassay). The relative alterations subsequent to variations in NaCl intake in S- and D-JGA renin activities as determined by radioimmunoassay or bioassay were comparable in magnitude and direction.

## DISCUSSION

These results confirm that the methods described by Dahlheim, Granger, and Thurau (1, 8) are capable of

accurately determining the renin activity of single JGA's. The major modification in this study in the system generating angiotensin using the renin contained within the single rat JGA was the substitution of a partially purified (12) heterologous (sheep) renin substrate for a highly purified (1, 8) homologous (rat) renin substrate. The observation that angiotensin generation was linear for up to 3 h of incubation suggests that this modification does not alter the basic characteristics of the assay system. A lower limit of sensitivity

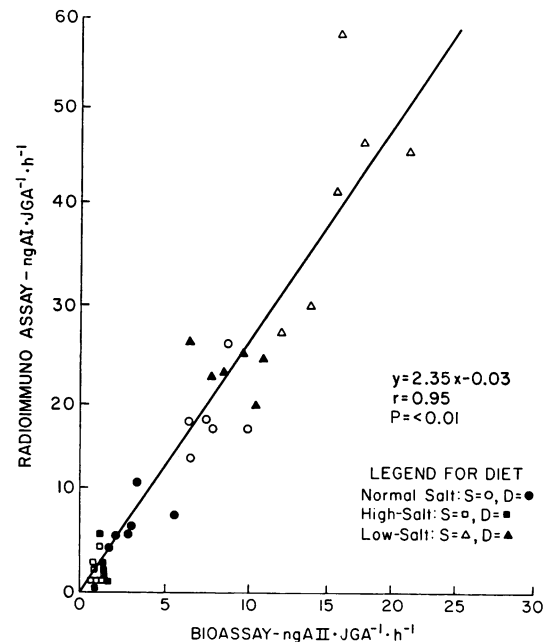


FIGURE 3 Comparison of JGA renin activity determined by bioassay of generated AII or radioimmunoassay of generated AI. S- and D-JGA's, three each, from rats on normal, high-, and low-NaCl diets (see text) were assayed.

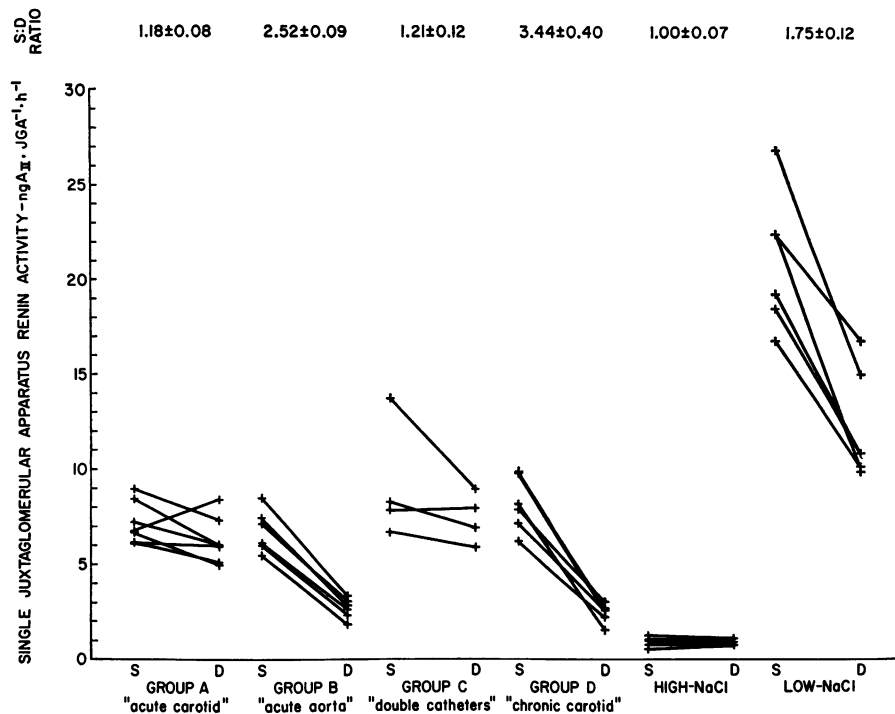


FIGURE 4 Comparison of (S- and D-JGA renin activities of all study groups. Each line connects the mean S- and D-JGA renin activities of a single rat. Groups A-D were on the regular NaCl diet. The S:D ratio is the ratio of the mean superficial to mean deep JGA renin activity for each group. The ratios obtained in groups A and C and after the high-salt diet are not significantly different from each other ( $P > 0.10$ ) but are significantly lower ( $P < 0.05$ ) than the ratios obtained in groups B and D and after the low-salt diet. See text for definition of groups and diets.

for the bioassay of angiotensin necessary for interpretation of JGA renin activity after NaCl loading or other maneuvers which may suppress JGA renin activity was established. In addition, the present study confirms the comparability of results obtained by using a biological (rat pressor response) assay of AII or radioimmunoassay of AI for quantifying generated angiotensin (15-17).

The mean JGA renin activity values observed by Granger et al. (1),  $14.0 \pm 15$  ng/JGA $\cdot 0.1$  ml $\cdot$ h<sup>3</sup> for superficial and  $13.4 \pm 1.6$  ng/JGA $\cdot 0.1$  ml $\cdot$ h for juxta-medullary JGA's, were higher than the mean values of  $7.22 \pm 0.40$  (S) and  $6.27 \pm 0.13$  ng AII $\cdot$ JGA<sup>-1</sup> $\cdot$ h<sup>-1</sup> (D) determined in similarly prepared rats in the present study (Table I, group A, "acute carotid"). This difference in absolute JGA renin activity values is presumably related to the lower sodium content (52 meq/kg) of the diet used in their studies (1, 8), rather

<sup>3</sup>The volume of renin substrate utilized has not been included in the units expressing renin activity in the present study or the report of de Rouffignac et al. (6) since activity is dependent on renin in the JGA. Granger et al. (1) have included substrate volume in the units, which are otherwise comparable.

than specific methodologic differences in the generation and/or measurement of angiotensin. In this comparably prepared group (Fig. 4, group A, "acute carotid") the ratio of S:D-JGA renin activity was  $1.18 \pm 0.08$ , not statistically different from the ratio of  $1.28 \pm 0.31$  ( $P > 0.70$ ) calculated with the results obtained by Granger et al. (1). This similarity in S:D-JGA renin activity suggests that the techniques used to micro-dissect S- and D-JGA's using silicone rubber injected into the carotid artery in the present study were comparable to both the arterial preparation (1) and the injection of Evans' blue dye into the jugular vein (1, 8) previously reported. It is apparent that cortico-medullary renin gradients were not observed in rats studied after manipulation of the cervical vasculature.

Many investigators avoid carotid artery manipulation because of the possible introduction of artifacts into the parameters under study. Logan, Jose, Eisner, Lilienfeld, and Slotkoff (18) utilized radioactive microspheres to evaluate intrarenal blood flow after hemorrhage in dogs. When a carotid artery was acutely occluded by inserting a catheter for the administration of microspheres a lower mean outer cortical renal blood flow rate and a higher mean inner cortical renal blood

flow rate was observed as compared to values obtained by using a brachial artery catheter for microsphere injection in normal control dogs. To avoid such possible artifacts, Wallin et al. (19) studied renal hemodynamics in rats using animals with a chronically implanted carotid artery catheter. It is of interest, in this regard, that previous direct studies of single glomerular renin content in rabbits performed without carotid artery or jugular vein manipulation by Brown et al. (3) and Gavras, Brown, Lever, and Robertson (20) demonstrated a renal cortical renin gradient, as observed in other species (2, 4, 5). In view of these observations additional studies were performed to evaluate the influence of surgical preparation on JGA renin activity and JGA renin gradients.

The results of these studies (Table I) suggest that the acute manipulation of the carotid artery, and not simply the injection of silicone-rubber into the carotid artery, was responsible for the lack of renin activity gradient in the rat in both the current study and that by Granger et al. (1). A significant gradient (Fig. 4) was observed in rats by using acute abdominal aorta catheterization (group B) or chronic carotid catheterization (group D). Similarly, an intrarenal cortical renin gradient in the rat was also observed by de Rouffignac et al. (6) using indirect techniques which did not require vascular manipulation. The results of de Rouffignac et al. (6) were obtained by analyzing the renin activity of cortical fragments and backcalculating glomerular renin activity using glomerular counts in cortical fragments. Therefore, comparisons of either the ratio of S to D renin activities or the absolute renin activities with the results of the present study cannot be made. While these marked differences in techniques do not allow direct comparisons with the present results, it is apparent that a corticomedullary renin gradient was present in rats studied without manipulation of the cervical vasculature.

The mechanism by which acute carotid artery manipulation results in loss of the intrarenal renin gradient is not apparent from the present study. An inverse relationship between carotid sinus pressure and renal sympathetic nerve activity has been demonstrated in the rabbit (21), cat (22-24), and dog (25-27). In addition to this inverse relationship, increased renal (sympathetic) nerve activity results in: increased renin-angiotensin system activity (28-30), which is unrelated to glomerular filtration (31) and is quantitatively more important at systemic blood pressures  $> 100$  mm Hg (32); and, increased renal vascular resistance (33-35). Alterations in intrarenal distribution of blood flow have also been observed after carotid manipulation (18, 35) or increased splanchnic nerve activity (35). Thus, as noted above, Logan et al. (18) observed an

alteration in the distribution of intrarenal blood flow determined by using radioactive microspheres because of acute carotid occlusion for the injection of microspheres. Pomeranz, Birtch, and Barger (35) similarly observed diminished outer cortical blood flow per gram of kidney and increased inner cortical + outer medullary flow per gram of kidney after carotid occlusion, baroreceptor denervation, and splanchnic nerve stimulation. The loss of an intrarenal renin gradient after carotid artery manipulation may be subsequent to similar alterations in intrarenal blood flow. Thus, increased blood flow to deeper cortical regions may inhibit renin release resulting in increased D-JGA renin content and renin activity, and decreased outer cortical flow may promote renin release resulting in decreased S-JGA renin content and renin activity. These predicted alterations in net JGA renin activity would also be modified by changes in renal renin synthesis as well as renin release, and these suggestions, as well as any inferences concerning the participation of these alterations in JGA renin activity in the elevated circulating renin levels observed after increased renal nerve activity in other studies, must be conjectural. Furthermore, the loss of intrarenal renin gradient after acute carotid manipulation may also be a direct result of altered and/or differing sympathetic discharge in S- and D-JGA's as a consequence of carotid sinus-related baroreceptor stimulation. The demonstration of adrenergic nerve endings applied to the various elements of the JGA (36-39) supports this possibility, and sympathetic nerve-related alterations in JGA renin activity may even result in the redistribution of cortical blood flow. Regardless of the exact mechanism, the S: D-JGA renin activity ratio observed in groups A ("acute carotid") and C ("double catheters") (Fig. 4) appears to represent an experimentally induced artifact, while those observed after acute abdominal aorta (group B) or chronic carotid artery (group D) catheterization (Fig. 4, groups B and D),  $2.52 \pm 0.09$  and  $3.44 \pm 0.90$ , respectively, are more representative of the basal state. The influence of other elements of the experimental preparation (e.g., anesthesia, open abdomen, etc.), which were similarly present in all experimental groups, was not evaluated.

To avoid the alterations induced by carotid artery manipulation all further studies were performed by using the abdominal aorta route. NaCl loading resulted in decreases in both S- and D-JGA renin activities, and NaCl deprivation resulted in increases in both S- and D-JGA renin activities (Table II), consistent with the well-known relationship between NaCl intake and renal renin content. All of the individual values obtained after the high NaCl intake were above the lower limit of sensitivity,  $0.5 \text{ ng AII} \cdot \text{JGA}^{-1} \cdot \text{h}^{-1}$ , and



may represent maximal decreases for the NaCl intake used in the present study. The low-NaCl diet had a higher potassium content than the regular NaCl diet. In view of the demonstrated inhibitory effect of potassium on the renin-angiotensin system (40) the elevations in JGA renin activity after NaCl deprivation might have been higher, relative to those observed on the regular NaCl diet, had a more comparable potassium intake been used.

Significant changes in the total renin activities of both S- and D-JGA's were observed (Table II) on the high- and low-NaCl diets. The absolute net decrease in S-JGA renin activity,  $5.84 \pm 0.11$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>, in animals on the high-NaCl diet was greater ( $P < 0.001$ ) than the absolute net decrease in D-JGA renin activity,  $1.73 \pm 0.06$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>. Similarly, after the low-NaCl diet the absolute net increase in S-JGA renin activity,  $14.28 \pm 1.47$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>, was greater ( $P < 0.01$ ) than the absolute net increase in D-JGA renin activity,  $9.62 \pm 1.19$  ng AI·JGA<sup>-1</sup>·h<sup>-1</sup>. These absolute net changes suggest that S-JGA's respond more than D-JGA's to alterations in NaCl intake. When the ratios of total S:D-JGA renin activities are compared this more marked absolute response of S-JGA's to altered NaCl intake is reflected by changes in the relative intrarenal renin gradient. Thus, the mean S:D-JGA renin activities (Fig. 4) after the high-NaCl diet,  $1.00 \pm 0.07$ , and the low-NaCl diet,  $1.75 \pm 0.12$ , are significantly different ( $P < 0.001$ ) from each other. These ratios are also significantly decreased as compared to the S:D-JGA renin activities observed in rats on a regular NaCl diet after abdominal aorta catheterization (group B,  $2.52 \pm 0.09$ ,  $P < 0.001$ ) or after chronic carotid artery catheterization (group D,  $3.44 \pm 0.40$ ,  $P < 0.005$ ). These relative changes in JGA renin activity, as reflected in the decreased S:D-JGA renin activity ratios observed, are consistent with an altered distribution in the cortical gradient of renin activity in response to altered NaCl intake.

The results obtained in the present study on rats on a low-NaCl diet cannot be compared to those obtained by Granger et al. (1), using a comparable low-NaCl diet, because of the probable artifact induced by acute carotid artery manipulation. While not directly comparable, as noted above, a more marked decrease in absolute superficial glomerular renin content after NaCl loadings for 3 wk was observed by de Rouffignac and co-workers (6).

#### ACKNOWLEDGMENTS

The authors wish to thank Drs. K. Thurau and H. Dahlheim for their assistance in acquiring the basic technique for single JGA renin analysis utilized in this study. The authors also acknowledge the secretarial assistance of Mrs. E. Foley.

#### REFERENCES

1. Granger, P., H. Dahlheim, and K. Thurau. 1972. Enzyme activities of the single juxtaglomerular apparatus in the rat kidney. *Kidney Int.* 1: 78-88.
2. Vikhert, A. M., and Y. A. Serebrovskaya. 1962. On the problem of localizing renin in the kidneys of normal animals and man. *Kardiologiya.* 2: 10-17.
3. Brown, J. J., D. L. Davies, A. F. Lever, R. A. Parker, and J. I. S. Robertson. 1965. The assay of renin in single glomeruli in the normal rabbit and the appearance of the juxtaglomerular apparatus. *J. Physiol. (Lond.)* 176: 418-428.
4. Horiuchi, K., H. Tanaka, K. Yamamoto, and J. Ueda. 1971. Distribution of renin in the dog kidney. *Life Sci.* 10: 727-734.
5. Rojo-Ortega, J. M., P. Granger, R. Boucher, and J. Genest. 1970. Studies on the distribution of the JGI in the renal cortex of dogs and beavers. *Nephron.* 7: 61-66.
6. de Rouffignac, C., J. P. Bonvalet, and J. Menard. 1974. Renin content in superficial and deep glomeruli of normal and salt loaded rats. *Am. J. Physiol.* 226: 150-154.
7. Rojo-Ortega, J. M., and J. Genest. 1970. Index de l'activite histochemique de la glucose-6-phosphate dehydrogenase dans la "macula densa" (IMD) et sa distribution dans le cortex renal chez le rat. *Pathol. Biol.* 18: 595-599.
8. Dahlheim, H., P. Granger, and K. Thurau. 1970. A sensitive method for determination of renin activity in the single juxtaglomerular apparatus of the rat kidney. *Pflügers Arch. Eur. J. Physiol.* 321: 303-315.
9. Horster, M., and K. Thurau. 1968. Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. *Pflügers Arch. gesamte Physiol. Menschen Tiere.* 301: 162-181.
10. Thurau, K. W. C., H. Dahlheim, A. Grüner, J. Mason, and P. Granger. 1972. Activation of renin in the single juxtaglomerular apparatus by sodium chloride in the tubular fluid at the macula densa. *Circ. Res. Suppl.* 2: 31: 182-186.
11. Snedecor, G. W., and W. G. Cochran. 1967. *Statistical Methods.* Iowa State University Press, Ames, Iowa. 6th edition. 593 pp.
12. Skinner, S. L. 1967. Improved assay methods for renin "concentration" and "activity" in human plasma. Methods using selective denaturation of renin substrate. *Circ. Res.* 20: 391-402.
13. Flamenbaum, W., T. A. Kotchen, and D. E. Oken. 1972. Effect of renin immunization on mercuric chloride and glycerol-induced renal failure. *Kidney Int.* 1: 406-412.
14. Haber, E., T. Koerner, L. B. Page, B. Kliman, and A. Purnode. 1969. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J. Clin. Endocrinol. Metab.* 29: 1349-1355.
15. Cohen, E. L., C. E. Grim, J. W. Conn, W. M. Blough, Jr., R. B. Guyer, D. C. Kem, and C. P. Lucas. 1971. Accurate and rapid measurement of plasma renin activity by radioimmunoassay. *J. Lab. Clin. Med.* 77: 1025-1038.
16. Sealey, J. E., J. Gerton-Banes, and J. Laragh. 1972. The renin system: variations in man measured by radioimmunoassay or bioassay. *Kidney Int.* 1: 240-253.

17. Menard, J., and K. J. Catt. 1972. Measurement of renin activity concentrations and substrate in rat plasma by radioimmunoassay of angiotensin I. *Endocrinology*. 90:422-430.
18. Logan, A., P. Jose, G. Eisner, L. Lilienfeld, and L. Slotkoff. 1971. Intracortical distribution of renal blood flow in hemorrhagic shock in dogs. *Circ. Res.* 29: 257-266.
19. Wallin, J. D., R. C. Blantz, M. A. Katz, V. E. Andreucci, F. C. Rector, Jr., and D. W. Seldin. 1971. Effect of saline diuresis on intrarenal blood flow in the rat. *Am. J. Physiol.* 221: 1297-1304.
20. Gavras, H., J. J. Brown, A. F. Lever, and J. I. S. Robertson. 1970. Changes of renin in individual glomeruli in response to variations of sodium intake in the rabbit. *Clin. Sci. (Oxf.)*. 38: 409-414.
21. Clement, D. L., C. L. Pelletier, and J. T. Shepherd. 1972. Role of vagal afferents in the control of renal sympathetic nerve activity in the rabbit. *Circ. Res.* 31: 824-830.
22. Ninomiya, I., N. Nisimaru, and H. Irisawa. 1971. Sympathetic nerve activity to the spleen, kidney and heart in response to baroreceptor input. *Am. J. Physiol.* 221: 1346-1351.
23. Wilson, M. F., I. Ninomiya, G. N. Franz, and W. V. Judy. 1971. Hypothalamic stimulation and baroreceptor reflex interaction on renal nerve activity. *Am. J. Physiol.* 221: 1768-1773.
24. Ninomiya, I., and H. Irisawa. 1969. Summation of baroreceptor reflex effects on sympathetic nerve activity. *Am. J. Physiol.* 216: 1330-1336.
25. Kezdi, P., and E. Geller. 1968. Baroreceptor control of postganglionic sympathetic nerve discharge. *Am. J. Physiol.* 214: 427-435.
26. Iriuchijima, J., and M. F. Wilson. 1968. Sympathetic vasoconstrictor activity to the kidney in carotid occlusion pressor reflex. *Proc. Soc. Exp. Biol. Med.* 131: 189-192.
27. Gilmore, J. P. 1964. Contribution of baroreceptors to the control of renal function. *Circ. Res.* 14: 301-317.
28. Mogil, R. A., H. Itskovitz, J. H. Russell, and J. J. Murphy. 1969. Renal innervation and renin activity in salt metabolism and hypertension. *Am. J. Physiol.* 216: 693-697.
29. Davis, J. O. 1971. What signals the kidney to release renin? *Circ. Res.* 28: 301-306.
30. Zehr, J. E., and E. O. Feigl. 1973. Suppression of renin activity by hypothalamic stimulation. *Circ. Res. Suppl. 1*. 32: 17-27.
31. Johnson, J. A., J. O. Davis, and R. T. Witty. 1971. Selective stimulation of renal nerves in the anesthetized dog. Effect of catecholamines and renal nerve stimulation on renin release in the nonfiltering kidney. *Circ. Res.* 29: 646-653.
32. LaGrange, R. G., C. H. Sloop, and H. E. Schmid. 1973. Elective stimulation of renal nerves in the anesthetized dog. Effect on renin release during controlled changes in renal hemodynamics. *Circ. Res.* 33: 704-712.
33. Vander, A. J. 1965. Effect of catecholamines and the renal nerves on renin secretion in anesthetized dogs. *Am. J. Physiol.* 209: 659-662.
34. Bunag, R. D., I. H. Page, and W. J. McCubbin. 1966. Neural stimulation of release of renin. *Circ. Res.* 19: 851-858.
35. Pomeranz, B. H., A. G. Birtch, and A. C. Barger. 1968. Neural control of intrarenal blood flow. *Am. J. Physiol.* 215: 1067-1081.
36. Ljungqvist, A., and J. Wägermark. 1970. The adrenergic innervation of intrarenal glomerular and extraglomerular circulatory routes. *Nephron*. 7: 218-229.
37. Friedberg, E. C. 1964. Distribution of the juxtaglomerular granules and the macula densa in the renal cortex of the mouse. *Lab. Invest.* 13: 1003-1013.
38. Hartroft, P. M. 1966. Electron microscopy of nerve endings associated with juxtaglomerular cells and macula densa. *Lab. Invest.* 15: 1127-1128A.
39. McKenna, O. C., and E. T. Angelakos. 1968. Adrenergic innervation of the canine kidney. *Circ. Res.* 22: 345-354.
40. Flamenbaum, W., T. A. Kotchen, R. Nagle, and J. S. McNeil. 1973. Effect of potassium on the renin-angiotensin system and HgCl<sub>2</sub>-induced acute renal failure. *Am. J. Physiol.* 224: 305-311.