

## Preliminary Observations on the Role of the Sympathetic Nervous System in Development and Maintenance of Experimental Renal Hypertension.\*† (31429)

LAWRENCE D. DORR AND MICHAEL J. BRODY

*Department of Pharmacology, University of Iowa College of Medicine, Iowa City*

Evidence indicating that the vasoconstrictor activity of angiotensin is dependent in part upon an intact sympathetic system(1,2) as well as suggestions for a possible neurogenic basis of chronic renal hypertension(3,4,5) have stimulated interest in the role of sympathetic innervation in the hemodynamic changes associated with renal hypertension. Studies demonstrating the disappearance of detectable blood levels of vasopressor material following the acute phase of renal hypertension have indirectly implicated a chronic neurogenic mechanism(6,7). The present study was undertaken in an attempt to determine directly whether the absence of the sympathetic nervous system influences development or maintenance of renal hypertension.

*Methods.* Experiments were performed on 53 female rats (Simonsen, Sprague-Dawley) separated into 4 groups: control(15), control with Goldblatt clamp(11), immunologically sympathectomized(11), and immunologically sympathectomized with Goldblatt clamp(16).

Immunological sympathectomy (ISX) was accomplished(8) by injection of anti-nerve growth factor (68,000 units/ml) for each of the first 14 days following birth. A dose equivalent to 1% of body weight was given subcutaneously every 24 hours.

When the rats attained an approximate weight of 150 g, they were placed in individual cages and conditioned daily in preparation for the handling necessary for blood pressure determinations. Blood pressures were recorded directly because of the inability to measure pressures in ISX rats by means of a tail cuff(8). At a weight of 200-250 g, the catheters for chronic measurement of blood pressure were positioned in the abdominal

aorta according to techniques reported previously by Weeks and Jones(9). During the same operation one renal artery was clamped using procedures described by Schaffenburg (10), and the contralateral kidney was removed. In this study the renal artery was constricted to a diameter of 0.015 inch. Following surgery, 60,000 units of penicillin G and 40 mg of dihydrostreptomycin were administered intramuscularly.

Bi- to tri-weekly readings of pulse pressure, mean blood pressure and heart rate were made beginning 5-7 days after surgery. To minimize movement, each rat was individually wrapped in a towel while its blood pressure was recorded. Pressures were recorded with a Satham P23G pressure transducer on an Offner direct writing oscillograph. Heart rate was determined by noting the number of pulse deflections per minute on the blood pressure tracing. Acute blood pressures (from carotid cannulation) were recorded under light ether anesthesia in those animals from which no chronic recording could be taken and from each rat surviving at termination of the experiment. At death or at the time acute blood pressures were recorded, the lumbar sympathetic chains were removed and examined histologically. In those rats which died before termination of the experiment, autopsies were performed to determine the cause of death.

Statistical analyses were performed by comparison of the means utilizing Duncan's new multiple range test with Kramer's modification for unequal replications(11) and by Student "t" test analyses described by Snedecor(12).

*Results.* The results, presented as average pressures for a week (mean of 2-3 readings) are summarized in Table I. In confirmation of recordings made under anesthesia(8), blood pressures of normal and immunologically sympathectomized rats were equivalent. Peak

\* Supported in part by a grant from the Iowa Heart Assn.

† A preliminary report of this study appeared in *Clinical Research*, 1964, v12, 362.

TABLE I. Time Course of Renal Hypertension in Normal (Control) and Immunologically Sympathectomized Rats.\*

Week†		Control hypertensive	Control	ISX hypertensive	ISX
1	S	127 ± 10 (9)	126 ± 9 (6)	125 ± 5 (11)	127 ± 6 (8)
	M	118 ± 10 (10)	112 ± 6 (9)	119 ± 5 (11)	118 ± 6 (8)
	D	117 ± 10 (9)	109 ± 7 (6)	112 ± 5 (11)	110 ± 5 (8)
2	S	139 ± 5 (10)	134 ± 8 (6)	158 ± 8 (11)	144 ± 5 (7)
	M	128 ± 4 (14)	123 ± 4 (7)	141 ± 6 (11)	124 ± 4 (7)
	D	122 ± 5 (10)	110 ± 5 (6)	126 ± 6 (11)	109 ± 4 (7)
3	S	167 ± 5 (7)	145 ± 5 (4)	181 ± 10 (5)	149 ± 4 (6)
	M	139 ± 4 (10)	127 ± 4 (7)	165 ± 9 (5)	132 ± 2 (7)
	D	121 ± 5 (7)	107 ± 10 (4)	151 ± 10 (5)	113 ± 3 (6)
4	S		147 ± 3 (2)	154 ± 14 (4)	150 ± 6 (4)
	M	144 ± 3 (4)	128 ± 5 (6)	139 ± 10 (5)	138 ± 4 (4)
	D		129 ± 5 (2)	116 ± 10 (4)	122 ± 9 (4)
5	S	151 ± 7 (3)	159 ± 7 (2)	139 ± 6 (3)	
	M	144 ± 5 (3)	135 ± 6 (4)	128 ± 8 (3)	
	D	132 ± 8 (3)	129 ± 5 (2)	115 ± 9 (3)	
6‡	S	163 ± 9 (6)		136 ± 15 (5)	105 ± 10 (4)
	M	140 ± 5 (7)	144 ± 11 (4)	125 ± 15 (5)	96 ± 10 (4)
	D	124 ± 4 (6)		105 ± 16 (5)	86 ± 10 (4)

\* Values represent weekly means ± standard error derived from average of 2-3 readings made in each rat. For each week the upper line is systolic pressure (S), the middle line mean pressure (M), and the lower line diastolic pressure (D). Figures in parentheses represent number of animals contributing to the average pressure value. Empty spaces indicate that no pressures were recorded. ISX, immunological sympathectomy.

† Week column designates the number of weeks following application of renal artery clamp.

‡ At termination of week 6, blood pressures of several rats were recorded under ether anesthesia (3 of 4 ISX, 6 of 7 clamped controls, and 4 of 5 clamped ISX rats).

pressure elevations were found in both clamped controls and clamped ISX rats during week 3. Systolic pressures of clamped controls were significantly different at this time only from controls ( $P < 0.02$ ). Systolic ( $P < 0.02$ ), mean ( $P < 0.01$ ) and diastolic ( $P < 0.01$ ) pressures of ISX with clamps were all higher than in ISX rats. The hypertension produced in the 2 groups appeared to be different in that hypertensive ISX rats had significantly higher mean ( $P < 0.02$ ) and diastolic ( $P < 0.02$ ) blood pressures than hypertensive controls. Systolic pressure of the 2 groups did not differ significantly. The systolic ( $P < 0.05$ ) and mean ( $P < 0.05$ ) blood pressures of the clamped ISX group returned to normotensive levels by week 5 while pressures of the clamped control group did not change significantly during this same period. The blood pressures of the hypertensive ISX group remained normotensive during week 6. It should be noted that pressures of the unclamped ISX rats remained stable at 4 weeks, a time when the decline in pressures of the clamped ISX rats was

established. There were no significant differences in pressures of these groups at 6 weeks.

Heart rate was also recorded and as presented in Table II is expressed as average weekly values. In the first week the ISX groups had significantly lower rates than the controls. The only other difference noted was in the third week when ISX rats without clamps had lower rates than the control group. Hypertension *per se* did not cause heart rates to differ.

Autopsies performed to determine the cause of death in animals which died prior to cessation of the experiment revealed that death was caused either by severe renal ischemia in clamped groups or abdominal hemorrhage which probably resulted from aortic damage.

Histological examination of the sympathetic nerves showed that administration of the anti-nerve growth factor resulted in essentially complete destruction of the sympathetic ganglion cells.

*Discussion.* The concept that renal hypertension results primarily from the release of

TABLE II. Heart Rates of Hypertensive and Normotensive Control and Immunologically Sympathectomized Rats.\*

Wk	Heart rate (beats/min)				C.V.,† %
	Control hyper- tensive	Control	ISX hyper- tensive	ISX	
1	<u>503</u> (8)	<u>503</u> (8)	<u>433</u> (11)	<u>388</u> (8)	11.1
2	<u>483</u> (12)	<u>463</u> (8)	<u>410</u> (11)	<u>421</u> (7)	12.4
3	<u>475</u> (9)	<u>457</u> (8)	<u>411</u> (5)	<u>395</u> (7)	14.2
4	<u>484</u> (4)	<u>423</u> (6)	<u>431</u> (4)	<u>420</u> (4)	11.5

\* Values represent weekly means derived from average of 2-3 readings made in each rat. Figures in parentheses represent number of animals. Data analyzed by Kramer's modification of Duncan's new multiple range test. Means underlined by same line are not significantly different. Means not underlined by the same line are significantly different ( $P < 0.05$ ). ISX, immunologically sympathectomized.

† C.V. = coefficient of variability.

renin and subsequent formation of the vasoconstrictor angiotensin(13,14,15,16) appears to be generally accepted. However, the lack of detectable amounts of vasoconstrictor material in chronic renal hypertension(6,7) implicates indirectly something other than a humoral mechanism for renal hypertension of long duration.

Increased activity of the sympathetic nervous system as the responsible agent for chronic renal hypertension has been suspected since the work of Ogden *et al*, which demonstrated that the administration of pentobarbital or yohimbine reduced the blood pressure of chronic hypertensive rats but did not affect the blood pressure of control rats(4). Recently Taquini precipitated a fall of blood pressure to equivalent levels in both normotensive and renal hypertensive animals by pithing(5). Laverty and Smirk found that acute sympathectomy reduced elevated resistance in the perfused limb of the renal hypertensive rat to the same level as that seen in the sympathectomized normotensive limb (3).

The possibility that the renin-angiotensin and sympathetic nervous systems may interact

is further supported by studies which demonstrate indirect actions of angiotensin. Zimmerman(2,17) has shown a significant reduction of the constrictor action of angiotensin following acute sympathectomy. Page and McCubbin(1) demonstrated enhanced pressor responses to tyramine and DMPP during angiotensin infusion. Bickerton and Buckley (15) showed that angiotensin can elevate blood pressure in part by central stimulation of sympathetic activity.

In the present study, treatment with the anti-nerve growth factor, which produces a functional sympathectomy(8), appeared to alter the course of renal hypertension. While sympathectomy did not affect the development of hypertension, the results at least suggest that hypertension of renal origin is not well maintained in the absence of functional sympathetic nerves. It is of interest to note that the onset of return to normotensive from hypertensive pressures coincides well in time with the disappearance of circulating vaso-pressor material, as reported in renal hypertensive rats(6) and dogs(7).

One observation made in this study might be of considerable importance to an understanding of the hemodynamic basis of renal hypertension. The control hypertensive animals developed only systolic hypertension, which suggested cardiac rather than vascular origin for the hypertensive state in these rats. As a means of estimating the validity of our observation, Dr. James R. Weeks, Upjohn Co., Kalamazoo, Mich. (who has had extensive experience with the methodology used in the present paper) studied pressure changes in a series of 6 control and 8 clamped, unilaterally nephrectomized rats, prepared in similar fashion to the animals used in this study. At the end of a 14-day observation period pressures in control animals were: systolic  $143 \pm 5$  (SE), mean  $124 \pm 3$ , diastolic  $112 \pm 2$ . The hypertensive rats showed the following pressures: systolic  $173 \pm 5$ , mean  $151 \pm 3$ , diastolic  $130 \pm 3$ . Compared to day 1, diastolic pressures increased  $1 \pm 2$  mm Hg in the controls and  $17 \pm 3$  mm Hg in the hypertensives. It thus appears likely that our observation was due to chance, and that diastolic pressure is elevated in renal hyper-

tension. The question which remains unanswered at present is whether or not immunologically sympathectomized rats develop more severe acute diastolic hypertension than do control animals.

It must be granted that our results were not completely satisfactory from a technical standpoint. In some animals no pressure was recorded, others died during the 6-week observation period, and finally, the level of hypertension achieved in the control animals was not great. Despite these drawbacks, these observations, considered in the light of the work of others, provide support for the postulate that the vasoconstrictor activity of sympathetic nerves is directly related to pathogenesis of the chronic phase of renal hypertension.

*Summary.* The influence of the sympathetic nervous system upon the course of experimental renal hypertension was examined in normal and immunologically sympathectomized rats. Blood pressures were recorded directly in the unanesthetized animal through indwelling catheters in the abdominal aorta. Immunological sympathectomy did not prevent the development of the acute phase of renal hypertension. Blood pressures of the hypertensive sympathectomized animals returned to normotensive levels 4-5 weeks following renal artery clamping, while untreated animals remained hypertensive at the termination of the experiment. It was concluded that the sympathetic nervous system is not required for development of renal hypertension, but may play a role in maintaining

the chronic phase of the hypertensive state.

The authors gratefully acknowledge the invaluable assistance provided by Richard A. Shaffer. Dr. R. K. Richards, Abbott Laboratories, North Chicago, Ill. graciously supplied the antiserum used in these experiments. Histological examination was performed by Dr. Louis M. Crews, Hazelton Laboratories, Inc., Falls Church, Va.

1. McCubbin, J. W., Page, I. H., *Circ. Res.*, 1963, v12, 553.
2. Zimmerman, B. G., *ibid.*, 1962, v11, 780.
3. Laverty, R., Smirk, H. F., *ibid.*, 1961, v9, 455.
4. Ogden, E., Collings, W. D., Saperstein, L. A., *Experimental Hypertension*, 1946, v3, 153.
5. Taquini, A. C., Jr., *Circ. Res.*, 1963, v12, 562.
6. Koletsky, S., Pritchard, W. H., *ibid.*, 1963, v13, 552.
7. Pritchard, W. H., Ormond, A. P., Jr., Koletsky, S., *Proc. Soc. Exp. Biol. and Med.*, 1964, v117, 127.
8. Brody, M. J., *Circ. Res.*, 1964, v15, 161.
9. Weeks, J. R., Jones, J. A., *Proc. Soc. Exp. Biol. and Med.*, 1960, v104, 646.
10. Schaffenburg, C. A., *ibid.*, 1959, v101, 676.
11. Steel and Torrie, *Principles and Procedures of Statistics*, Ed. 1, 1960.
12. Snedecor, G. W., *Statistical Methods*, Ed. 5, 1956.
13. Gollan, F., Richardson, E., Goldblatt, H., *J. Exp. Med.*, 1948, v88, 389.
14. Skeggs, L. T., Jr., Kahn, J. R., Shumway, N. P., *ibid.*, 1952, v95, 241.
15. Robertson, J. I. S., *J. Physiol.*, 1963, v166, 27P.
16. Haas, E., Goldblatt, H., *Am. J. Physiol.*, 1964, v207, 1077.
17. Zimmerman, B. G., Gomez, J., Stitzel, R. E., *Pharmacologist*, 1964, v6, 175.

Received June 13, 1966. P.S.E.B.M., 1966, v123.

### Studies on Lymphopoiesis. VII. Size Distribution of Bovine Thoracic Duct Lymphocytes.\* (31430)

C. R. SIPE, A. D. CHANANA, E. P. CRONKITE, D. D. JOEL, AND L. M. SCHIFFER

*Medical Research Center, Brookhaven National Laboratory, Upton, N. Y.*

Virchow observed lymphocytes within the lymph in 1851(1). Many investigators have since attempted to determine their origin,

function, life span and fate. Lymphocytes were classified into categories based upon diameter, morphology and staining characteristics. A common classification separated them into the 3 divisions of small, medium and large. It was difficult, however, to estab-

\* Research supported by U. S. Atomic Energy Commission.