The Vascular Reactivity to Vasoactive Substances and the Electrolyte Contents in Arterial Walls

By

Hiroshi Demura, Soitsu Fukuchi, Hidenori Takahashi
and Koji Goto

From the Department of Internal Medicine, Tohoku University School of Medicine, Sendai; Director: Prof. T. Torikai

(Received for publication, May 6, 1965)

The reactivity of blood vessels to DL-noradrenaline and angiotensin II was examined using the perfusion technic of isolated hindlimbs of rats and rabbits. Rats with Goldblatt hypertension and animals treated with DOCA, angiotensin II and NaCl; with D-aldosterone 21-monoacetate, angiotensin II and NaCl for 4 weeks; and with angiotensin II were found to show an increased reactivity to vasoactive substances, an increase in the vascular wall potassium content and decrease in serum potassium concentration. Rats treated with aminonucleoside and with carbon tetrachloride had a decreased reactivity and a decreased potassium content of their arterial walls. Vascular reactivity was elevated in the hindlimbs of rabbits perfused with the solution containing a high concentration of potassium, while it was almost normal when the solution containing a low concentration of potassium was used. The changes in reactivity of the hindlimb vessels did not always parallel the blood pressure changes.

These results show that the changes in the intracellular potassium concentration seem to be more important determinants of the changes in the reactivity of the hindlimb vessels to vasoactive agents than the changes in the extracellular potassium concentration.

It is generally recognized that the reactivity of blood vessels to vasoactive agents is elevated in hypertension. Some investigators have shown that this hyperreactivity is closely related to changes in the concentration of electrolytes of the vessel walls. For further clarification of the basis for the hyperreactivity of blood vessels in hypertension, we have used isolated perfused hindlimbs of animals. These isolated hindlimbs were used in order to avoid neurogenic and humoral vasoconstrictive influences.¹

MATERIALS AND METHODS

Rats

Sixty-eight adult male Wistar rats weighing about 200 g each were utilized

for the following experiments:

1. *Normal controls.* Ten normal male rats were used for this experiment.
2. *Goldblatt hypertension.* Hypertension was produced as described previously.² Eight animals were killed 10 weeks after clamping of the left renal artery.
3. *DOCA, angiotensin II and NaCl.* Desoxycorticosterone acetate (DOCA) pellets, 16.7 mg each, were implanted in 5 animals. Two weeks later, injections of angiotensin II of 50 μ g per 100 mg of body weight suspended in peanut oil were started and given daily for 2 weeks. A one per cent solution of NaCl was used for the drinking water.
4. *Aldosterone, angiotensin II and NaCl for 4 weeks.* Nine animals were injected intramuscularly with D-aldosterone 21-monoacetate, 50 μ g per 10 g of body weight, and angiotensin II, 50 μ g per 100 g of body weight. These injections were given once daily for 4 weeks.
5. *Angiotensin II.* Five animals were given angiotensin II subcutaneously, 50 μ g per 100 g of body weight, daily for 2 weeks.
6. *Aldosterone, angiotensin II and NaCl for 2 weeks.* Five animals were given the same amount of D-aldosterone 21-monoacetate and angiotensin II using the same method as in (4) but the injections were continued for 2 weeks only.
7. *Aldosterone and NaCl.* Nine animals were given D-aldosterone 21-monoacetate subcutaneously, 50 μ g per 100 g of body weight, once daily for 2 weeks.
8. *Hydrochlorothiazide.* Five animals were given hydrochlorothiazide subcutaneously, 10 mg per 100 g of body weight, once daily for 2 weeks.
9. *Aminonucleoside.* Six animals were given 2.5 mg of aminonucleoside subcutaneously once daily for 10 days. Edema and ascites occurred after the treatment in these animals.
10. *Carbon tetrachloride.* Six animals were given subcutaneous injections of carbon tetrachloride, 0.1 ml per 100 g of body weight, mixed with the same amount of fluid paraffin twice weekly for 10 weeks. After the treatment, edema and ascites occurred and the liver changed histologically to cirrhotic.

Rabbits

Eighteen normal adult male rabbits weighing about 3 kg each were utilized for the following experiments using a low and high concentrations of potassium in the infusing solution.

1. *Normal potassium concentration.* Ten male rabbits were used for this experiment. The hindlimbs of these rats were perfused with solutions containing 5.7 mEq/l of potassium.
2. *High potassium concentration.* The hindlimbs of 4 animals were perfused with solutions containing a high concentration of potassium (11.4 mEq/l).
3. *Low potassium concentration.* The hindlimbs of 4 animals were perfused with solutions containing a low concentration of potassium (1.9 mEq/l).

Arterial pressure was measured using an ear artery by the method of Watson³ in rabbits and using a tail plethysmograph in rats. The sodium and potassium contents of the aorta were measured as described previously.² Both the adrenals and kidneys were examined histologically. Paraffin sections of the adrenal glands were stained with hematoxylin-eosin and frozen sections were stained for lipid with scarlet-red hematoxylin. The reactivity of blood vessels to vasoactive substances was assessed by means of isolated hindlimb preparations (Fig. 1). Heparin was injected intravenously before surgery. In rats, the abdominal

Fig. 1 Schema of the estimation of vascular reactivity by means of isolated hindlimbs preparation in rats and rabbits.

aorta was exposed and cannulated in a downward direction. The vessels of the hindquarters were perfused with Locke's solution (potassium concentration 5.9 mEq/l). In rabbits, the vessels of single hind leg were perfused after cannulation of one common iliac artery. Injection of DL-noradrenaline and angiotensin II were made into the tubing connected to the arterial cannula. The volumes of injections were all 0.5 ml. The reactivity of the blood vessels of the hindlimbs to vasoactive agents was estimated by measuring the decrease in the flows of the perfusing fluid under a constant pressure of perfusing solution. The rate of flow of perfusing fluid was measured by counting drops of the solution as it emerged from the inferior vena cava. The decrease of flow after the injections of vasoactive agents was calculated as follows.

$$\text{Decrease of flow} = \frac{\text{Drops during 3 minutes before injection} - \text{drops during 3 minutes after injection}}{\text{Drops during 3 minutes before injection}}$$

RESULTS

The results are summarized in Tables I and II.

Rats

1. *Normal rats.* The mean systolic blood pressure in 10 male normal rats was 82 mmHg. The mean sodium content in the aorta was 218.3, and the mean potassium content 87.3 mEq per kg dry tissue.

2. *Goldblatt hypertension.* The elevation in arterial pressure was most marked in this experiment. Both the sodium and potassium content of the aortic wall were increased ($p < 0.01$) (Fig. 2). The reactivity of the hindlimb vessels to DL-noradrenaline and angiotensin II was elevated (Figs. 3 and 4). The adrenal weights were increased. Hypertrophy and increased lipid content of the cortical cells were conspicuous in the outermost part of the fasciculata. Slight hypertrophy and swelling of cells were also observed in the glomerulosa.

Fig. 2. Sodium and potassium contents in the aorta of rats.

3. *DOCA, angiotensin II and NaCl.* A slight elevation of blood pressure was observed. Serum sodium concentration was elevated, and serum potassium concentration was decreased. Both sodium and potassium contents of the aorta

TABLE I. Changes of Blood Pressure, Electrolyte Contents in the Aortic Wall and Histological Findings of Adrenal Gland in Experimental Animals

Treatment	No.	Blood pressure mmHg	Aortic wall			Adr. weight %	Adrenal gland			
			Na mEq/Kg	K mEq/Kg			Glom.	Subgl.	Fasc.	
						Body weight			Outer	Inner
Normal controls	10	82±2	218.3±4.3	87.3±3.6		14.8	→	→	→	→
Goldblatt hypertension	8	146±7	240.9±5.8*	112.8±6.8*		25.6	↗	→	↗	→
DOCA, angiotensin II and NaCl	5	116±5	342.2±13.5*	142.4±16.0*		19.2	↘	→	↑	↗
Aldosterone, angiotensin II and NaCl (4 W)	9	128±4	273.1±23.7*	123.6±9.0*		23.4	↗	↑	↗	↗
Angiotensin II	5	84±2	232.4±13.6†	107.0±12.3†		18.9	↗	↑↑	↗	↗
Aldosterone, angiotensin II and (2 W) NaCl	5	140±6	238.2±7.1*	111.0±9.7 ^z		16.0	↗	↗	↗	→
Aldosterone, and NaCl	9	76±4	224.5±6.7‡	84.6±7.1		17.2	↗	↗	↗	→
Hydrochlorothiazide	5	78±3	225.5±16.0*	93.4±6.8*						
Aminonucleoside	6	80±2	322.0±6.6*	67.7±4.2*			→	→	↗	→
Carbon tetrachloride	6	77±5	254.4±5.0*	64.7±5.0*			→	→	↑↑	↗

* p<0.01 † 0.01<p<0.05 ‡ 0.05<p<0.1

TABLE II. Changes of Vascular Reactivity in Experimental Animals

Treatment	No.	Decrease of flow by angiotensin (%)				Decrease of flow by DL-noradrenaline (%)			
		2 ng	10 ng	50 ng	100 ng	0.3 μ g	1.0 μ g	3.0 μ g	5.0 μ g
Rats									
Normal controls	10	10.2 $\pm$ 6.8	19.1 $\pm$ 3.9	32.5 $\pm$ 5.2		10.4 $\pm$ 5.2	18.8 $\pm$ 7.4	29.7 $\pm$ 6.4	
Goldblatt hypertension	8	16.9 $\pm$ 11.0	35.6 $\pm$ 11.0	57.2 $\pm$ 8.0		16.9 $\pm$ 7.8	35.6 $\pm$ 8.9	55.4 $\pm$ 21.6	
DOCA, angiotensin II and NaCl (4 W)	5	21.0 $\pm$ 11.2	48.1 $\pm$ 15.2	61.7 $\pm$ 12.4		16.8 $\pm$ 1.5	54.6 $\pm$ 8.3	73.4 $\pm$ 13.9	
Aldosterone, angiotensin II and NaCl (4 W)	9	15.6 $\pm$ 12.1	44.6 $\pm$ 15.4	55.1 $\pm$ 17.1		26.2 $\pm$ 7.2	42.5 $\pm$ 8.8	63.8 $\pm$ 12.4	
Angiotensin II	5	10.1 $\pm$ 2.8	30.7 $\pm$ 9.3	64.0 $\pm$ 11.1					
Aldosterone, angiotensin II and NaCl (2 W)	5	16.9 $\pm$ 4.4	20.8 $\pm$ 0.8	25.1 $\pm$ 3.0		19.8 $\pm$ 5.1	25.6 $\pm$ 8.1	32.0 $\pm$ 12.6	
Aldosterone and NaCl	9	11.3 $\pm$ 2.5	18.5 $\pm$ 3.4	32.5 $\pm$ 8.6		15.7 $\pm$ 4.7	19.8 $\pm$ 4.9	22.0 $\pm$ 8.1	
Hydrochlorothiazide	5	10.8 $\pm$ 2.8	15.5 $\pm$ 5.2	24.5 $\pm$ 8.0		11.0 $\pm$ 3.0	18.6 $\pm$ 3.3	22.3 $\pm$ 2.8	
Aminonucleoside	6	5.2 $\pm$ 2.2	11.0 $\pm$ 2.2	16.3 $\pm$ 4.3	19.7 $\pm$ 2.2	5.4 $\pm$ 2.3	9.5 $\pm$ 4.3	14.8 $\pm$ 4.2	18.5 $\pm$ 1.6
Carbon tetrachloride	6	7.6 $\pm$ 1.9	11.9 $\pm$ 2.6	16.5 $\pm$ 2.4	21.0 $\pm$ 1.9	9.9 $\pm$ 3.0	12.3 $\pm$ 4.5	16.7 $\pm$ 4.6	21.6 $\pm$ 2.3
Rabbits									
Normal K concentration	10	10.3 $\pm$ 3.5	27.7 $\pm$ 5.2	39.3 $\pm$ 6.3		13.1 $\pm$ 6.5	25.9 $\pm$ 5.8	36.8 $\pm$ 9.3	
High K concentration	4	14.6 $\pm$ 7.9	55.8 $\pm$ 12.1	74.4 $\pm$ 9.8		19.5 $\pm$ 9.6	47.1 $\pm$ 17.5	56.3 $\pm$ 13.0	
Low K concentration	4	4.0 $\pm$ 0	22.8 $\pm$ 7.1	29.0 $\pm$ 9.0		12.9 $\pm$ 2.6	27.3 $\pm$ 2.2	30.8 $\pm$ 4.9

Fig. 3. Changes in vascular reactivity by various treatments in rats.

- (1) Normal rats (2) Goldblatt hypertension
 (3) DOCA, angiotensin II and NaCl
 (4) Aldosterone, angiotensin II and NaCl for 4 weeks
 (5) Angiotensin II (6) Aldosterone, angiotensin II and NaCl for 2 weeks
 (7) Aldosterone and NaCl (8) Hydrochlorothiazide
 (9) Aminonucleoside (10) Carbon tetrachloride

were markedly increased ($p < 0.01$). The reactivity of the hindlimb vessels to DL-noradrenaline and angiotensin II was marked in this experimental group. The adrenal weights were increased. Coarse droplets of lipid were observed in the glomerulosa, while the width of this layer was decreased. In the outermost part of the fasciculata, hypertrophy and increased cell lipids were seen.

4. *Aldosterone, angiotensin II and NaCl for 4 weeks.* Arterial pressure was slightly elevated. Both the sodium and potassium contents of the aorta were greatly increased ($p < 0.01$). The reactivity of the hindquarter vessels to vaso-active agents and the adrenal weights were remarkably increased. Coarse droplets of lipid were found in the glomerulosa. A marked hyperplasia and an increased number of mitosis were seen in the cortical cells of the subglomerulosa. Clear swollen cells were conspicuous in the outer part of the fasciculata.

5. *Angiotensin II.* Arterial pressure was not elevated. Both potassium and sodium contents of the aortic walls were slightly increased (sodium 0.05 $p < 0.1$, potassium $0.01 < p < 0.05$). The reactivity of the hindquarter vessels to

Fig. 4. Changes in vascular reactivity by various treatments in rats.

- (1) Normal rats (2) Goldblatt hypertension
- (3) DOCA, angiotensin II and NaCl
- (4) Aldosterone, angiotensin II and NaCl for 4 weeks
- (6) Aldosterone, angiotensin II and NaCl for 2 weeks
- (7) Aldosterone and NaCl (8) Hydrochlorothiazide
- (9) Aminonucleoside (10) Carbon tetrachloride

angiotensin II was markedly elevated. The adrenal weights were increased. Hypertrophy and occasional mitosis of the cortical cells were observed in the glomerulosa, in the subglomerulosa and in the outer part of the fasciculata. The lipid content of the cells was increased in the glomerulosa, and decreased in the outer part of the fasciculata.

6. *Aldosterone, angiotensin II and NaCl for 2 weeks.* Arterial pressure was elevated. Both the sodium and potassium content of the aorta were increased ($p < 0.01$). The reactivity of the hindquarter vessels remained unchanged to DL-noradrenaline, while it was slightly decreased to angiotensin II. The adrenal weights were increased slightly. The histological changes were about the same as those found in the animals of (3), but were less conspicuous.

7. *Aldosterone and NaCl.* Arterial pressure was not elevated. The electrolyte contents in the aortic wall and the reactivity of the hindlimb vessels remained within the normal range. The adrenal weights were slightly increased. Atrophy

and a decrease in the lipid content of the cortical cells were seen in the glomerulosa. The width of the subglomerulosa was markedly decreased. Slight atrophy and coarse lipid droplets were observed in the cells of the outer part of the fasciculata.

8. *Hydrochlorothiazide*. Arterial pressure remained unchanged. The serum potassium concentration was slightly decreased, while the sodium concentration was normal. The electrolyte content of the aortic wall was remained unchanged. The reactivity of the hindlimb vessels was slightly decreased both to DL-noradrenaline and to angiotensin II.

9. *Aminonucleoside*. Arterial pressure was normal. The sodium content of the aorta was markedly increased, while the potassium content was moderately decreased ($p < 0.01$). The reactivity of the hindlimb vessels to DL-noradrenaline and to angiotensin II was decreased. The adrenal weights were slightly increased.

10. *Carbon tetrachloride*. Arterial pressure was normal. The sodium content of the aorta was slightly increased, while the potassium content was moderately decreased ($p < 0.01$). The reactivity of the hindlimb vessels was markedly decreased both to DL-noradrenaline and to angiotensin II. The adrenal weights

Fig. 5. Changes in vascular reactivity in the hindlimbs of rabbits perfused with solutions containing a high or low concentration of potassium.

- (1) Normal potassium concentration ($K=5.7$ mEq/l)
- (2) High concentration of potassium ($K=11.4$ mEq/l)
- (3) Low concentration of potassium ($K=1.9$ mEq/l)

Fig. 6. Changes in vascular reactivity in the hindlimbs of rabbits perfused with solutions containing a high or low concentration of potassium.

- (1) Normal potassium concentration ($K=5.7\text{mEq/l}$)
- (2) High concentration of potassium ($K=11.4\text{ mEq/l}$)
- (3) Low concentration of potassium ($K=1.9\text{ mEq/l}$)

were greatly increased. Histological findings of the adrenals in the animals of (8) and (9) were about the same as reported previously.²

No histological abnormalities were found in the kidneys of any of the animals in these studies.

Rabbits (Figs. 5 and 6)

1. *Normal potassium concentrations.* The vascular reactivity of the hindlimb vessels in ten normal male rabbits was slightly higher than in the normal male rats.

2. *High potassium concentration.* The reactivity of the hindlimb vessels to angiotensin II was markedly increased. The reactivity to DL-noradrenaline was moderately increased.

3. *Low potassium concentration.* The reactivity of the hindlimb vessels to DL-noradrenaline was in the normal range. The reactivity to angiotensin II was slightly decreased.

DISCUSSION

Certain structural and chemical factors have been reported to be responsible for the increased reactivity of blood vessel to vasoactive agents in patients and in animals with hypertension.⁴ The structural changes consist of reductions of the lumen of the arteries together with hypertrophy of the smooth muscle of the arterial walls. There have been several reports about the chemical changes of the vascular walls in experimental hypertensive animals. Recently Hollander *et al.*⁶ observed the changes in the reactivity of the blood pressure relating to the bindings of the catecholamines in the tissues and postulated that one of the basic disturbances in essential hypertension might be an accumulation of active DL-noradrenaline in the arterioles. Waugh⁷ reported that changes in neither sodium nor chloride ion were essential for alteration of the excitation process of arterial smooth muscle. A change in the potassium content of the blood vessel walls has been believed to be the most important chemical factor responsible for a change in vascular reactivity.^{4,8-11} With one exception, all of the results of the present study support the conclusion on the role of potassium.

In our examination of the isolated perfused hindlimb vessels, increased reactivities to DL-noradrenaline and angiotensin II were observed in rats with Goldblatt hypertension; rats treated with DOCA, angiotensin and NaCl; rats treated with aldosterone, angiotensin and NaCl for 4 weeks; and rats treated with angiotensin. In all of these groups of experimental animals, both the sodium and potassium contents of the aortic wall were increased. On the contrary, a decreased reactivity to DL-noradrenaline and angiotensin II was observed in rats with aminonucleoside nephrosis and in rats with carbon tetrachloride liver cirrhosis. In these experimental groups, the sodium content of the aorta was increased, while the potassium content was decreased. Therefore it may be concluded that the variation of reactivity of the isolated hindlimb vessels to vasoactive agents is directly related to the potassium content of the walls of the blood vessels and is independent of variation in the sodium content of the walls.

In rats treated with aldosterone, angiotensin and NaCl for 2 weeks, the reactivity of the hindlimb vessels to DL-noradrenaline and angiotensin II was not increased in spite of the increases in the sodium and potassium contents of the aorta. This is inexplicable at present. In rats treated with aldosterone and NaCl, the reactivity of the hindlimb vessels to vasoactive substances was within the normal range consistent with the normal potassium content of the aortic wall.

McQueen¹² reported that isolated perfused hindlimb vessels of rats with renal and renoprival hypertension had increased responsiveness to noradrenaline. He found a good correlation between the height of the blood pressure and the noradrenaline response. In our experiment, hyperreactivity of the hindlimb vessels was accompanied by a blood pressure elevation in rats with Goldblatt

hypertension; in rats treated with DOCA, angiotensin and NaCl; and in rats treated with aldosterone, angiotensin and NaCl for 4 weeks. But in rats treated with aldosterone, angiotensin and NaCl for 2 weeks, no increase in the reactivity of the hindlimb vessels was observed in spite of a marked blood pressure elevation. On the other hand, hyperreactivity was observed in normotensive rats treated with angiotensin. The decrease in reactivity to angiotensin was also observed in normotensive rats treated with hydrochlorothiazide. Thus the changes in reactivity of the hindlimb vessels did not always parallel the changes in blood pressure. NollaPanades¹³ reported that the increase in DL-noradrenaline response found in the hindlimb vessel was not the result of the hypertension, but was in some way related to the mechanism that produced the hypertension.

What mechanism has changed the potassium content of blood vessels? Kaplan and Silah¹⁴ observed a decreased sensitivity to angiotensin II in patients with secondary aldosteronism such as liver cirrhosis, in patients with malignant hypertension and in patients with renovascular hypertension. They concluded that the sensitivity to angiotensin II is closely related to the circulating renin level. Laragh¹⁵ and Johnston and Jose¹⁶ reported that in patients with secondary aldosteronism, the responsiveness of blood pressure was depressed to angiotensin II, but it was normal to DL-noradrenaline. In our examination of the hindlimb vessels, however, the reactivity to DL-noradrenaline as well as to angiotensin II was decreased in rats with aminonucleoside nephrosis and in rats with carbon tetrachloride liver cirrhosis. Furthermore, in rats treated with angiotensin II, the reactivity of the hindlimb vessels to angiotensin II was markedly increased. Therefore our findings do not support their impression that the response to angiotensin II is regulated by the circulating angiotensin level. Kaplan and Silah stated that angiotensin II sensitivity test can be used for the differential diagnosis of the renovascular hypertension and essential hypertension. But, again, in our rats with Goldblatt hypertension, angiotensin II response was increased.

Besides the renin-angiotensin-aldosterone system, adrenal glucocorticoids have been known to induce an increase in the aortic wall potassium content.^{11,12,17} In rats potassium administration increases the width of the zona glomerulosa.¹⁷ In our studies, adrenal weights and the width of the fasciculata were increased in animals with increased reactivity of the isolated hindlimb vessels. But there seemed to be no close relation between the reactivity of the hindlimb vessels and the width of the glomerulosa. Thus an adrenal etiology for the changes in the potassium content of the vessel walls was not suggested by the histological findings in the glands.

In our recently reported clinical studies, the variation of DL-noradrenaline and angiotensin II sensitivities was directly related to the serum potassium concentration.¹⁹ Similarly in the present experimental studies, a decreased reactivity

to DL-noradrenalin and angiotensin II was observed in rats with a decreased serum potassium concentration, without change in the electrolytes of the aorta, following treatment with hydrochlorothiazide.^{20,21} The reactivity of the hindlimb vessels of rabbits to DL-noradrenaline and angiotensin II was markedly increased, when the vessels were perfused with solutions containing a high concentration of potassium. In contrast, when the vessels were perfused with solutions containing a low concentration of potassium, the reactivity of the vessels to DL-noradrenalin was in the normal range, while vascular reactivity to angiotensin II was slightly decreased. These observations are consistent with those of Dodd and Daniel²² and Lastz.²³ However, in animals treated with DOCA, the response of the hindlimb vessels to vasoactive agents was markedly elevated in spite of the very low serum potassium concentration. Thus the changes in the extracellular potassium concentration seem to be less important determinants of changes in the reactivity of the hindlimb vessels to vasoactive agents than the changes in the intracellular potassium concentration.

In the present experiment, the normal reactivity of the hindlimb vessels to vasoactive substances was observed in rats treated with aldosterone and NaCl, while the increased reactivity was observed in rats with angiotensin; with DOCA, angiotensin and NaCl; and with aldosterone, angiotensin and NaCl for four weeks. Conn *et al.*²⁴ described that excessive production of angiotensin plus excessive aldosterone production leads eventually to malignant hypertension, while the primary production of excessive amount of aldosterone with its tendency to depress the production of angiotensin does not result in malignant hypertension. Masson *et al.*²⁵ also have supported the hypothesis that high blood levels of renin and aldosterone may have some pathogenic significance in clinical malignant hypertension. Therefore, the normal vascular reactivity to vasopressor substances is probably one of the reasons why a patient with excessive aldosterone and hypertension of long term duration seldom has a severe vascular damage.

Acknowledgment

The authors are greatly indebted to Dr. Franz Gross of the CIBA in Switzerland for a supply of D-aldosterone 21-monoacetate and angiotensin II. We also would like to express thanks to Dr. Nobuaki Sasano, Assistant Professor of Pathology in Tohoku University School of Medicine, for his advice on histological studies and to Prof. Tatsuo Torikai and Prof. Koroku Hashimoto, Department of Pharmacology, Tohoku University School of Medicine, for their leadership throughout this study.

The help of Dr. Charles A. Nugent, Associate Professor of Medicine, University of Utah, College of Medicine, in editing this manuscript is acknowledged.

References

- 1) McQueen, E.G. *Clin. Sci.*, 1961, **21**, 133.
- 2) Fukuchi, S., Hanata, M., Takahashi, H., Demura, H. & Torikai, T. *Tohoku J. exp. Med.*, 1964, **84**, 125.

- 3) Watson, E.M. *J. Lab. clin. Med.*, 1926-7, **12**, 706.
 - 4) Bevan, J.A. & Osher, J.V. *Circulat. Res.*, 1963, **13**, 346.
 - 5) Reldeaf, P.D. & Tobian, L. *ibid.*, 1958, **6**, 185.
 - 6) Hollander, W., Yagi, S. & Kramsch, D.M. *Circulation*, 1964, **30** (Suppl. II), 1.
 - 7) Waugh, W.H. *Circulat. Res.*, 1962, **11**, 264.
 - 8) Tobian, L. & Reldeaf, P.D. *Amer. J. Physiol.*, 1958, **192**, 325.
 - 9) Bohr, D.F. & Conlet, P.L. *Amer. J. Cardiology*, 1961, **9**, 700.
 - 10) Barr, L., Headings, V.E. & Bohr, D.F. *J. Gen. Physiol.*, 1962, **46**, 19.
 - 11) Freed, S.C., St. George, S. & Rosenman, R.H. *Circulat. Res.*, 1959, **7**, 219.
 - 12) McQueen, E.G. *Clin. Sci.*, 1956, **15**, 523.
 - 13) Nolla-Panades, J. *Circulat. Res.*, 1963, **12**, 3.
 - 14) Kaplan, N.M. & Silah, J.H. *J. clin. Invest.*, 1964, **43**, 659.
 - 15) Laragh, J.H. *Circulation*, 1962, **25**, 203.
 - 16) Johnston, C.I. & Jose, A.D. *J. clin. Invest.*, 1963, **42**, 1411.
 - 17) Ledingham, J.H. *Clin. Sci.*, 1954, **13**, 543.
 - 18) Denae, H.W., Shaw, J.H. & Greep, R.O. *Endocrinology*, 1948, **43**, 133.
 - 19) Fukuchi, S., Hanata, M., Takahashi, H., Demura, H. & Goto, K. *Tohoku J. exp. Med.*, 1964, **85**, 181.
 - 20) Plummer, A.J. & Yonkman, F.F. *Amer. J. Cardiology*, 1960, **5**, 640.
 - 21) Freis, E.D., Wnako, A., Schnaper, H.W. & Frohlich, E.D. *J. clin. Invest.*, 1960, **39**, 1277.
 - 22) Dodd, W.A. & Daniel, E.E. *Circulat. Res.*, 1960, **8**, 451.
 - 23) Lastz, L. *Nature*, 1960, **185**, 696.
 - 24) Conn, J.W., Knopf, R.F. & Nesbit, R.M. *Aldosterone*, Oxford, Blackwell Scientific Publications, 1964, p. 327.
 - 25) Masson, G.M.C., Mikasa, A. & Yasuda, H. *Endocrinology*, 1962, **71**, 505.
-