*Br. J. Pharmac. Chemother.* (1968), **33**, 136–144.

# THE EFFECTS OF HYDROCHLOROTHIAZIDE AND FRUSEMIDE ON NORADRENALINE SENSITIVITY AND BLOOD PRESSURE OF SALT-LOADED RATS BEFORE AND AFTER NEPHRECTOMY

BY

MARY F. LOCKETT AND T. E. NICHOLAS

*From the Department of Pharmacology, University of Western Australia, Nedlands, Western Australia*

(*Received December* 1, 1967)

The benzothiadiazine diuretics were introduced by Novello & Sprague (1957) 10 years ago and although their hypotensive action was discovered within the year (Hollander & Wilkins, 1957) it remains unexplained. It was at first assumed that these drugs were hypotensive in man by consequence of their diuretic action because the initial fall in the resting blood pressure of hypertensive patients was accompanied by reduction in plasma volume, cardiac output and plasma concentration of sodium (Freis, Wanko, Wilson & Parrish, 1958; Fuchs, Moyer & Newman, 1960). After approximately 5 days of continuous therapy with a benzothiadiazine, however, refractoriness to the diuretic effect develops; plasma volume, cardiac output and plasma electrolytes are restored, but the hypotensive action remains (Gifford, Mattox, Orvis, Sones & Rosenvear, 1961; Talso & Carballo, 1960; Freis *et al.*, 1958; Fuchs *et al.*, 1960; Conway & Lauwers, 1961). The similarity of the diuretic effects of these compounds in normal and hypertensive patients (Hollander, Chobanian & Wilkins, 1960) contrasts with their ability to lower the blood pressure solely in the hypertensives (Freis *et al.*, 1958; Friedman, Nakashima & Friedman, 1960) and again stresses the dissociation of these two actions.

Because the hypotensive action of the benzothiadiazines is accompanied by reduction in vascular sensitivity to adrenaline and noradrenaline (Beavers & Blackmore, 1958) it is possible that these drugs influence plasma concentrations of a substance which exerts some control over the bore of resistance vessels. The experiments recorded in this paper constitute an initial test of this hypothesis. The influence of hydrochlorothiazide on the sensitivity of the systemic vascular bed to noradrenaline has been compared in normal and nephrectomized rats under pentobarbitone.

## METHODS

Male Wistar rats, weighing 180–240 g, were housed in open wire mesh cages in an air-conditioned room at 24° C; a pellet diet containing 1% NaCl (Lockett & Nail, 1965), or a similar diet containing 0.5% NaCl and tap water were constantly available. All operations and experiments were carried out under anaesthesia induced by intraperitoneal injection of sodium pentobarbitone, 45 mg/kg. Bilateral nephrectomy was performed through a single dorsal skin incision. The kidneys were separately approached by muscle splitting, the adrenal glands were gently freed from the upper

pole before retroperitoneal mobilization of the kidney, ligature of the ureter and hilar vessels by a single purse-string suture and excision of the kidney. Muscle and skin layers were separately sutured.

*Measurements*

Mean systemic arterial pressure was recorded from a heparinized P.E.50 polythene cannula in the right common carotid artery which was connected to a mercury manometer in all but the last two experiments. In these experiments a force-displacement transducer (E. & M. Instrument Co. Inc., Houston, Texas), coupled to a Heathkit pen-recorder replaced the mercury manometer. Cardiac output was determined by application of the direct Fick principle. Arterial and right ventricular blood samples were obtained by cannulation of the right common carotid artery and right external jugular vein, respectively, in heparinized rats (1,000 u./rat intravenously). The method of Watts & Gourley (1953) was used to measure oxygen consumption: blood oxygen was estimated by means of a Beckman oxygen electrode set in a 0.2 ml. cuvette within a constant temperature water bath and coupled to a Beckman Physiological Gas Analyzer. Heart rates were determined from electrocardiographs obtained from needle electrodes leading through a coupler (Type 9857) to a Beckman RT Dynograph, using an amplifier Type 474A. Concentrations of sodium and potassium in plasma were determined by means of an EEL flame photometer and of chloride by electrometric titration (Willard & Boldyreff, 1929). Spontaneous changes in plasma volume were followed by duplicate capillary haematocrit readings made on carotid arterial blood samples. In one series of experiments plasma volume was increased by infusion of freshly prepared heparinized rat plasma, 3 ml./rat.

*Drugs*

Sodium pentobarbitone (Veterinary Nembutal, Abbott Laboratories Ltd.), heparin (Evans Medical Ltd.), L-noradrenaline and hydrochlorothiazide (6-chloro-7-sulphamyl-3,4-dihydro-1,2,4-benzothiadiazine, 1,1-dioxide) (Ciba Laboratories Ltd.) were obtained commercially. Frusemide (Furosemide 4-chloro-*N*-(2-furylmethyl)-5-sulphamoyl anthranilic acid) was received as a gift from Hoechst Pharmaceuticals Ltd. The diuretics were in tablet form and were administered as suspensions by stomach tube. Noradrenaline was freshly dissolved in 0.9% NaCl for intravenous injections (0.05–0.15 ml.) washed in with 0.05 ml. of 0.9% NaCl.

*Design of experiments*

Animals to be used in each experiment were divided into groups of comparable weight. A pretreatment was randomly assigned to each group. Because each experiment necessarily extended over several weeks, pretreatments were staggered so that equal numbers of animals from each group were examined each day. Control animals invariably received mock-treatments. Two doses of noradrenaline were selected for continuous use after establishment of their dose-effect curves in preliminary experiments or the pressor effect of intravenous injections. The chosen doses regularly produced submaximal effects which lay on the long linear section of the log dose-effect curves. These doses were altered in the last experiment for which higher sensitivity isometric transducer recording was used.

*Statistical analysis*

Differences between the mean observation derived from the various groups were examined by Fisher's *t* test.

## RESULTS

### *Effect of hydrochlorothiazide and frusemide on the resting mean arterial pressure of mildly hypertensive rats, normal and nephrectomized*

Normal rats rendered mildly hypertensive by a diet containing approximately 1% of salt exhibited significantly lowered mean arterial blood pressures ($P<0.05$) 3 hr after the oral administration of hydrochlorothiazide 2 mg/rat or frusemide 40 mg/rat (Fig. 1). The

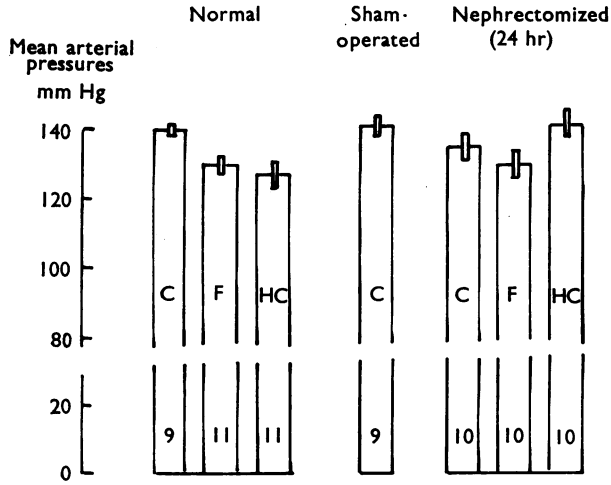


Fig. 1. Resting mean arterial pressures of normal, sham-operated and nephrectomized rats, untreated and after oral administration of frusemide or hydrochlorothiazide. The height of each rectangle shows the mean value of the resting arterial blood pressure under pentobarbitone anaesthesia in mm Hg: the standard errors and the numbers of animals used are indicated by the inset rectangles and numerals, respectively. Key: control animals (mock-treated), C; 2 hr after frusemide (40 mg/rat), F; 3 hr after hydrochlorothiazide (2 mg/rat), HC.

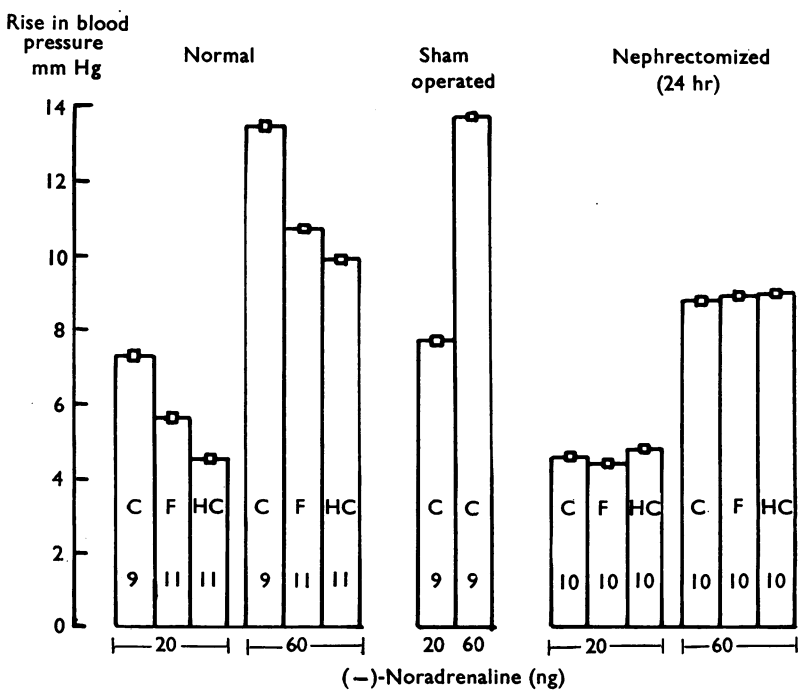


Fig. 2. Pressor actions of (−)-noradrenaline in normal, sham-operated and nephrectomized rats, untreated and after oral administration either of frusemide (40 mg/rat) or hydrochlorothiazide (2 mg/rat). The height of each rectangle shows the mean rise in arterial pressure in response to intravenous injections of either 20 or 60 ng of (−)-noradrenaline: standard errors and the numbers of animals inset, and key, as in Fig. 1. Ordinate: responses in mm Hg; abscissa: ng of (−)-noradrenaline, as indicated.

effects of these two diuretics on mean arterial pressure did not differ significantly. By contrast, neither diuretic caused significant change in the resting mean arterial pressures of these mildly hypertensive animals 24 hr after nephrectomy (Fig. 1).

*Effect of hydrochlorothiazide on the pressor response to noradrenaline in mildly hypertensive rats*

Figure 2 shows pressor responses to (−)-noradrenaline 20 and 60 ng intravenously in mildly hypertensive rats. The pressor effects of noradrenaline were very significantly reduced 2 hr after frusemide 40 mg, and even more so 3 hr after hydrochlorothiazide. Because reductions in the pressor responses to the high and low doses were proportional, both diuretics caused a simple shift of the log-dose effect curves to the left and did not cause significant changes in slope. The pressor actions of noradrenaline were reduced 24 hr after nephrectomy ($P<0.001$) but were unaltered in mock-operated animals. Nephrectomy caused a shift to the left in the log dose-effect curve without significant change in slope. Neither frusemide nor hydrochlorothiazide altered the sensitivity of nephrectomized rats to noradrenaline. Overall, the reduction in sensitivity to noradrenaline caused by hydrochlorothiazide 2 mg did not differ markedly from the desensitization to noradrenaline caused by nephrectomy. Figure 3 shows that desensitization of the systemic vascular bed to noradrenaline is apparent 1.5 hr after oral

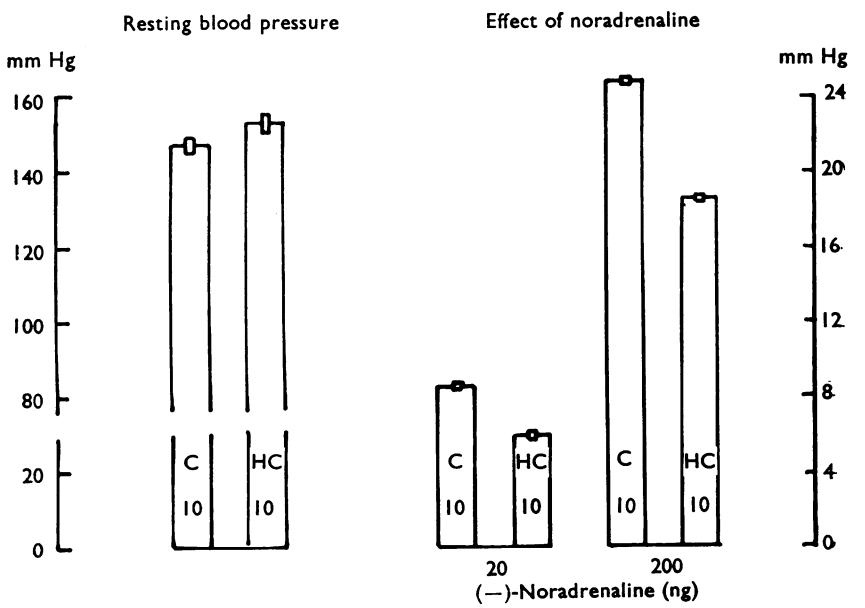


Fig. 3. Influence of hydrochlorothiazide (2 mg/rat) on the pressor effect of noradrenaline. The height of each rectangle shows the mean resting arterial pressure (left) or rise in pressure in response to 20 and 200 ng of (−)-noradrenaline, intravenously (right): standard errors and numbers of animals inset and key as in Fig. 1. Ordinate: mm Hg; abscissa (right only): ng of noradrenaline.

administration of hydrochlorothiazide. Because the resting mean arterial pressure is still unaffected at 1.5 hr but has fallen at 3 hr (compare Figs. 2 and 3), these two phenomena are dissociated in onset.

*Effect of hydrochlorothiazide on plasma volume and electrolytes, cardiac output and heart rate, in rats*

The haematocrit values, cardiac outputs and concentrations of sodium, potassium and chloride in plasma were unaffected 3 hr after the oral administration of hydrochlorothiazide 2 mg (Table 1). This pretreatment did, however, significantly lower the heart rate.

TABLE 1

HAEMATOCRIT VALUES, PLASMA ELECTROLYTES, CARDIAC OUTPUT AND HEART RATE 3 HR AFTER ORAL ADMINISTRATION OF EITHER 2 MG HYDROCHLOROTHIAZIDE OR SUSPENSION BASE ALONE TO HYPERTENSIVE RATS

The values shown are means±standard errors: the figures within brackets show the numbers of animals examined. Two asterisks signifies $P<0.01$.

| Parameter | Treatment | |
|---|---|---|
| | Suspension base | Hydrochlorothiazide |
| Body weight (g) | 232·1±5·80 (8) | 232·5±6·55 (8) |
| Haematocrit (cells %) | 42·1±0·83 (18) | 42·9±0·79 (18) |
| Cardiac output (ml./min) | 37·7±4·9 (9) | 35·9±3·0 (9) |
| Heart rate (beats/min) | 389·2±5·9 (11) | 361·6±6·5 (10)** |
| Plasma electrolytes: | | |
| Na+ (m-equiv/l.) | 142·0±1·54 (6) | 142·1±1·29 (6) |
| K+ (m-equiv.l.) | 4·8±0·22 (9) | 4·3±0·13 (9) |
| Cl′ (m-equiv/l.) | 98·6±2·62 (6) | 93·3±2·23 (6) |

TABLE 2

HAEMATOCRIT VALUES AND PLASMA ELECTROLYTES IN RATS 24 HR AFTER SHAM-OPERATIONS OR NEPHRECTOMY

The values shown are means±their standard errors. Two asterisks signify $P<0.01$.

| | Sham-operated | Nephrectomized |
|---|---|---|
| Sodium (m-equiv/l.) | 142·0 ±1·54 (6) | 140·6 ±0·18 (6) |
| Potassium (m-equiv/l.) | 4·30±0·28 (6) | 5·29±0·24 (6)** |
| Chloride (m-equiv/l.) | 98·6 ±2·62 (6) | 94·3 ±1·20 (6) |
| Haematocrit (cells %) | 40·2 ±0·66 (5) | 34·3 ±0·93 (5)** |

Table 2 demonstrates that the concentration of potassium in the plasma has risen ($P<0.01$) and the haematocrit value has fallen ($P<0.001$) 24 hr after nephrectomy. Figure 4 shows that an increase in plasma potassium by approximately 2 m-equiv/l. resulting from an oral dose of potassium chloride 30 mg administered 1.5 hr before an experiment, caused significant increase (not decrease) in the vascular sensitivity of mildly hypertensive rats to noradrenaline. Figure 5 demonstrates that expansion of the plasma volume such that the haematocrit value fell by 8 to 11% enhanced the pressor actions of noradrenaline and did not antagonize reduction in sensitivity to noradrenaline caused by hydrochlorothiazide 2 mg 3 hr after its administration (compare Figs. 5 and 2).

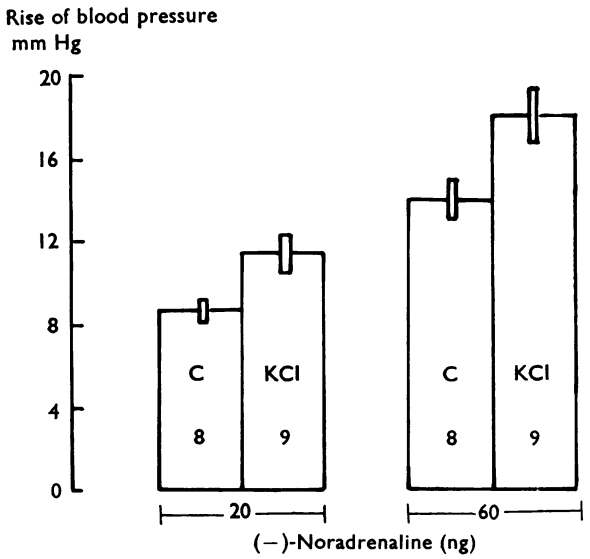


Fig. 4. Effect of elevation of the plasma potassium concentration on the pressor response to noradrenaline. The height of each rectangle shows the mean rise in arterial pressure in response to intravenous injections of either 20 or 60 ng of (−)-noradrenaline: standard errors and the number of animals are inset. Key: control rats; C: elevated plasma concentration of potassium, KCl.

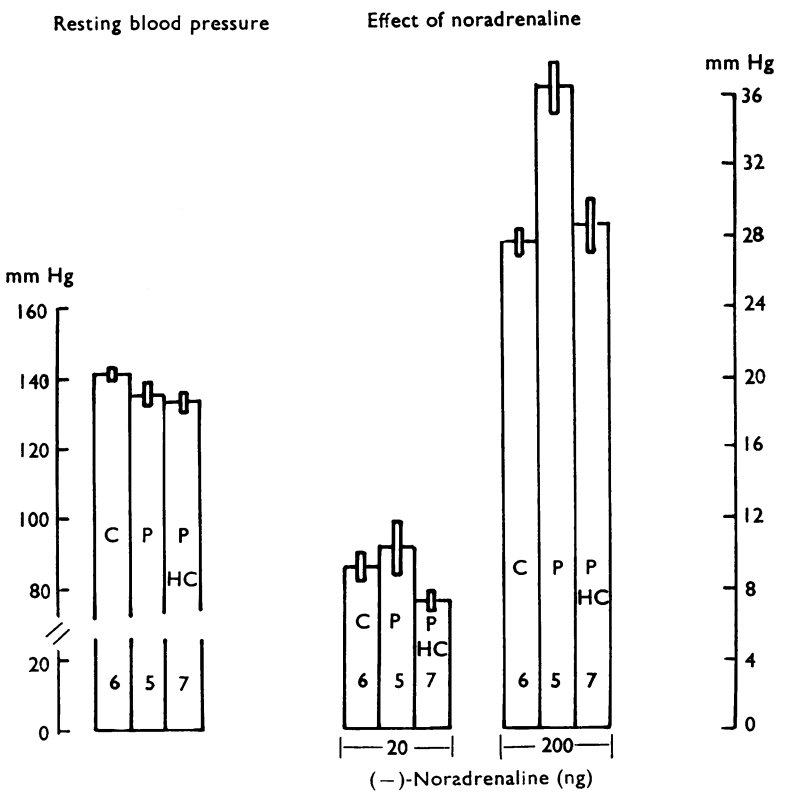


Fig. 5. Effect of expansion of plasma volume on the desensitization to noradrenaline caused by hydrochlorothiazide (2 mg/rat). The height of each rectangle shows the mean resting arterial pressure (left) or rise of pressure in response to 20 and 200 ng of (−)-noradrenaline intravenously (right). Standard errors and numbers of animals inset as in Fig. 1. Ordinates: mm Hg; abscissa: as Fig. 3. Key: Control animals, C; plasma volume expanded by intravenous injection of fresh rat plasma 3 ml./animal, P; plasma volume similarly expanded but 3 hr after hydrochlorothiazide, PHC.

## DISCUSSION

The antihypertensive actions of hydrochlorothiazide (Conway & Lauwers, 1960) and of frusemide (Lindner, 1966) in man are well known. Hydrochlorothiazide has also been shown to lower the resting blood pressure of rats made hypertensive by treatment with both deoxycorticosterone acetate and a high salt intake (Friedman *et al.*, 1960) but there are no prior reports available of the antihypertensive effect either of hydrochlorothiazide or of frusemide in rats made hypertensive by administration of salt alone. Whereas the benzothiadiazines lower the blood pressure solely in hypertensive subjects (Freis *et al.*, 1958) they depress vascular sensitivity to catecholamines in both hypertensive and normotensive man (Freis *et al.*, 1958), in normotensive dogs (Beavers & Blackmore, 1958) and in normotensive rats (Cession, 1964). This action of the benzothiadiazines has now been demonstrated for hydrochlorothiazide and for frusemide in rats made hypertensive solely by chronic salt loading.

There are three further points of interest. First, marked desensitization of the systemic vascular bed to the pressor action of noradrenaline was evident 1.5 hr after an oral dose of hydrochlorothiazide. This phenomenon preceded both the antihypertensive (compare Figs. 3 and 2) and the natriuretic (Cohen & Lockett, unpublished) actions of the drug by more than 1 hr. Hence the vascular desensitization caused by the drug has been dissociated in time course from its antihypertensive effect.

Second, the antihypertensive effect of hydrochlorothiazide developed synchronously with the natriuretic action of this drug, but before the induced diuresis had produced changes in plasma volume, plasma electrolytes and cardiac output (Table 1). This observation accords with many previous demonstrations of dissociation between the antihypertensive actions of the benzothiadiazines and their diuretic effects, for the antihypertensive action of the benzothiadiazines persists in patients made refractory to their diuretic action by their uninterrupted use (Gifford *et al.*, 1961 ; and many others). The bradycardia caused by hydrochlorothiazide in rats is probably a consequence of desensitization to noradrenaline because a tonic vagal influence on the pacemaker is not demonstrable in rats under pentobarbitone anaesthesia (unpublished observation).

Finally, absence both of the antihypertensive and of the anti-noradrenaline actions of hydrochlorothiazide has been demonstrated in salt-hypertensive rats 24 hr after nephrectomy. In this post-operative phase the resting mean arterial pressure was still unchanged but sensitivity to noradrenaline had decreased spontaneously, perhaps fortuitously, to the extent caused by treatment of unoperated rats with hydrochlorothiazide. Both the plasma volume and the plasma potassium level had risen 24 hr after nephrectomy. However, both increase in plasma volume and increase in plasma potassium enhanced, and did not decrease, the dual vascular actions of hydrochlorothiazide in sham-operated salt-hypertensive rats. It is therefore concluded that both the anti-noradrenaline and the anti-hypertensive actions of hydrochlorothiazide are dependent on renal function.

Treatment with hydrochlorothiazide has been found to increase the granularity of the juxtaglomerular apparatus (Tobian, Janecek, Foker & Ferreira, 1962) and indirect correlation between the granularity of this apparatus and the rate of renin secretion has been shown (Tobian, 1960). These observations and the synchronous onset of the anti-

hypertensive and natriuretic effects of hydrochlorothiazide suggest that change in the intraluminal concentration of sodium at the level of the macula densa (Thurau, 1964) may possibly have mediated the antihypertensive effects of hydrochlorothiazide in these acute experiments by reducing the rate of secretion of renin. It is, however, necessary to postulate a direct inhibitory effect of hydrochlorothiazide on the secretion of renin if the anti-noradrenaline action of this diuretic is to be interpreted in terms of the renin-angiotensin system, because the anti-noradrenaline action of hydrochlorothiazide precedes its diuretic effect by more than 1 hr. Interplay between the pressor effects of angiotensin and of noradrenaline is undoubted although the nature of this interplay is complex, in dispute and in part species dependent (Laurence & Nagle, 1963 ; Laverty, 1963 ; Bickerton & Buckely, 1961 ; Feldberg & Lewis, 1964 ; White & Ross, 1966 ; Hughes, 1968). Yet direct inhibition of renin secretion by hydrochlorothiazide and hence of the formation of angiotensin in plasma (Peart, 1960) could possibly account for the anti-noradrenaline actions of this duretic.

## SUMMARY

1. The actions of single oral doses of hydrochlorothiazide 2 mg or of frusemide 40 mg on resting mean arterial pressure and on vascular sensitivity to noradrenaline have been examined under pentobarbitone anaesthesia in salt-hypertensive rats.

2. Both diuretics cause reduction in vascular sensitivity to noradrenaline and a fall in mean arterial pressure.

3. Reduction in vascular sensitivity to noradrenaline induced by hydrochlorothiazide precedes, by more than 1 hr, the fall in mean arterial pressure and the natriuresis caused by the drug.

4. Both these vascular actions of hydrochlorothiazide are absent 24 hr after nephrectomy at a time when mean arterial pressures are unchanged: plasma sodium and chloride are unaltered, plasma volume and potassium are slightly but significantly raised.

5. An increase in plasma volume did not antagonize the vascular actions of hydrochlorothiazide.

6. An increase in the plasma potassium concentration potentiated the actions of noradrenaline.

The expenses of this work were defrayed by a grant from the Australian Universities Research Grants Committee.

## REFERENCES

BEAVERS, W. R. & BLACKMORE, W. P. (1958). Effect of chlorothiazide on vascular reactivity. *Proc. Soc. exp. Biol. Med.*, **98**, 133–135.

BICKERTON, R. K. & BUCKELY, J. P. (1961). Evidence for a central mechanism in angiotensin induced hypertension. *Proc. Soc. exp. Biol. Med.*, **106**, 834–836.

CESSION, G. (1964). Effet de la chlorothiazide sur les activités de quelques agents hypertenseurs chez le rat. *C. r. Séanc. Soc. Biol.*, **158**, 1186–1188.

CONWAY J. & LAUWERS, P. (1960). Haemodynamic and hypotensive effects of long-term therapy with chlorothiazide. *Circulation*, **21**, 21–27.

FELDBERG, W. & LEWIS, G. P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. *J. Physiol., Lond.*, **171**, 98–108.

FREIS, E. D., WANKO, A., WILSON, I. M. & PARRISH, A. E. (1958). Chlorothiazide in hypertensive and in normotensive patients. *Ann. N.Y. Acad. Sci.*, **71**, 450–455.

FRIEDMAN, S. M., NAKASHIMA, M. & FRIEDMAN, C. L. (1960). Relation of saluretic and hypotensive effects of hydrochlorothiazide in rats. *Am. J. Physiol.*, **198**, 148–152.

FUCHS, M., MOYER, J. H. & NEWMAN, B. E. (1960). Human clinical pharmacology of the newer diuretics. *Ann. N.Y. Acad. Sci.*, **88**, 795–808.

GIFFORD, R. W., MATTOX, V. R., ORVIS, A. L., SONES, D. A. & ROSENVEAR, J. W. (1961). Effect of thiazide diuretics on plasma volume, body electrolytes and excretion of aldosterone in hypertension. *Circulation*, **24**, 1197–1205.

HOLLANDER, W., CHOBIAN, A. V. & WILKINS, R. W. (1960). The role of diuretics in the management of hypertension. *Ann. N.Y. Acad. Sci.*, **88**, 975–986.

HOLLANDER, W. & WILKINS, R. W. (1957). Chlorothiazide: a new type of drug for the treatment of arterial hypertension. *Boston med. Qt.*, **8**, 69–75.

HUGHES, I. E. (1968). An investigation of the effects of angiotensin on the release of neurohumoral transmitters at motor adrenergic and cholinergic nerve terminals. *J. Pharm. Pharmac.*, **20**, 116–124.

LAURENCE, D. R. & NAGLE, R. E. (1963). The effects of bretylium and guanethidine on the pressor responses to noradrenaline and angiotensin. *Br. J. Pharmac. Chemother.*, **21**, 403–413.

LAVERTY, R. (1963). A nervously-mediated action of angiotensin in anaesthetized rats. *J. Pharm. Pharmac.*, **15**, 63–68.

LINDNER, E. (1966). Fursemide. *Arzneimittel-Forsch.*, **16**, 628–630.

LOCKETT, M. F. & NAIL, B. (1965). Propylthiouracil modifies the urinary effects of growth hormone and of aldosterone in rats. *J. Physiol., Lond.*, **176**, 371–377.

NOVELLO, F. C. & SPRAGUE, J. M. (1957). Benzothiadiazines as novel diuretics. *J. Am. Chem. Soc.*, **79**, 2028–2029.

PEART, W. S. (1965). The renin-angiotensin system. *Pharmac. Rev.*, **17**, 143–172.

TALSO, P. J. & CARBALLO, A. J. (1960). Effects of benzothiadiazines on serum and total body electrolytes. *Ann. N.Y. Acad. Sci.*, **88**, 822–840.

THURAU, K. (1964). Renal hemodynamics. *Am. J. Med.*, **38**, 698–719.

TOBIAN, L. (1960). Interrelationship of electrolytes, juxtaglomerular cells and hypertension. *Physiol. Rev.*, **40**, 280–312.

TOBIAN, L., JANACEK, J., FOKER, J. & FERRIERA, D. (1962). Effect of chlorothiazide on renal juxtaglomerular cells and tissue electrolytes. *Am. J. Physiol.*, **202**, 905–908.

WATTS, D. T. & GOURLEY, D. R. H. (1953). A simple apparatus for determining the basal metabolism of small animals in a student laboratory. *Proc. Soc. exp. Biol. Med.*, **84**, 585–588.

WHITE, F. N. & ROSS, G. (1966). Adrenal dependent circulatory responses to angiotensin in the cat. *Am. J. Physiol.*, **210**, 1118–1121.

WILLARD, H. H. & BOLDYREFF, A. W. (1929). A simple reference electrode for potentiometric titrations. *J. Am. Chem. Soc.*, **51**, 471–473.