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# Adaptation to the natriuretic actions of hydrochlorothiazide, frusemide and amiloride, by rats

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Rats treated thrice daily with maximally natriuretic doses of amiloride or of frusemide showed a markedly diminished natriuretic response (adapted) by the third day and complete adaptation to hydrochlorothiazide by the fifth day of treatment. Normal rats supplied with 0·7% NaCl as drinking fluid and adrenalectomized rats maintained by either a high salt intake or by injections of doca could not adapt to the natriuretic effects of hydrochlorothiazide. There was no adaptation to the effects of these diuretics on $K^+$ excretion. Treatment of rats for eight successive days with amiloride, frusemide or hydrochlorothiazide caused significant elevation of the juxtaglomerular index and hyperactivity in the zona glomerulosa of the adrenal. Treatment for eight days with spironolactone did not produce these changes. The *in vitro* production of aldosterone was significantly increased in glands taken from animals pretreated for eight days with hydrochlorothiazide: spironolactone did not influence this synthesis. Plasma concentrations of corticosterone were unaffected by eight days of exposure to hydrochlorothiazide, were raised by this exposure to frusemide and were lowered by amiloride. It is considered that the renin-angiotensin-aldosterone system plays a rôle in adaptation, over days, to the natriuretic effects of sulphonamide and probably other natriuretic diuretics, in the rat.

The renal excretion of sodium, potassium and chloride increases when thiazide diuretics are first administered to man (Wilkins, Hollander & Chobanian, 1958; Freis, 1959; Heinemann, Demartini & Laragh, 1959). In consequence, the extracellular fluid volume and the cardiac output decrease (Hollander, Chobanian & Wilkins, 1959; Villarreal, Exaire & others, 1962), there is over-activity in the sympathetic nervous system (Tobian, 1967) and plasma concentrations of renin increase (Bourgoignie, Cantazaro & Perry, 1968). However, when thiazides are administered daily, their diuretic action diminishes, a positive salt balance appears and gradually restores the extracellular fluid volume and the cardiac output to near normal or normal values (Gifford, Mattox & others, 1961; Talso & Carballo, 1960; Freis, Wanko & others, 1958; Conway & Lauwers, 1961). This adaptation to the natriuretic action of the thiazides is generally attributed (Brown, Davis & Johnston, 1966; Bourgoignie & others, 1968) to an increase in the secretion of aldosterone (Fraser, James & others, 1965) caused by the raised plasma concentration of renin, for the enzyme renin interacts in the plasma with its $\alpha_2$-globulin substrate to release a decapeptide angiotensin I which is rapidly converted, principally in lung, to angiotensin II (Peart, 1965). Angiotensin II enhances the rate of secretion of aldosterone from the zona glomerulosa of the adrenal (Kaplan, 1965).

Numerous experiments, recently reviewed (Vander, 1967), have demonstrated that the secretion of renin can most certainly be influenced by extra-renal factors, but there

is evidence that an intrarenal mechanism exists additionally for the regulation of renin secretion. Thurau (1966) has suggested that the macula densa may act as a sensor of change in the load or in the concentration of sodium passing the luminal margin of these cells to regulate the secretion of renin. The object of the present work was to attempt an assessment of the importance of this intrarenal regulation of renin secretion in the process of adaptation to the natriuretic actions of diuretic agents. Measurements of rates and extent of adaptation to natriuretic drugs with sites of action proximal to and near the macula densa (frusemide and hydrochlorthiazide, respectively: Seldin, Eknoyan & others, 1966) and distal to the macula densa (Amiloride; Baba, Lant & others, 1968) have, therefore, been compared, in rats. (Meng, 1967; Crabbé, 1968; Brenner, Keimowitz & others, 1969; Lant, Baba, & Wilson, 1967.)

## METHODS

Female Wistar rats, 150–200 g, were fed a standard pellet diet (Wesfarmers Ltd) containing an average of 10 mequiv sodium per 100 g and drank tap water unless otherwise stated. The animals were housed in a single air-conditioned room at 23–25° in which all experiments were conducted, and were accustomed to handling, stomach tubes and injections before use.

Bilateral adrenalectomy was performed under methohexital sodium (40 mg/kg, i.p.) anaesthesia by the method of Zarrow, Yochim, & others (1964). These animals were maintained post-operatively either by administration of deoxycorticosterone acetate (doca, 250 $\mu$g/100 g body weight per day, i.m. in olive oil) or by substitution of aqueous 0·9% NaCl for drinking water, and were not used before the eleventh post-operative day.

*Collections of urine* were made thrice weekly during determinations of dose and time-effect curves and in the week before measurement of the rate of adaptation to natriuretic action. Collections were also made on the first, third, fifth and either the seventh or the eighth day of continuous treatment. All urine collections constituting a single experiment were made at the same time of day, as described by Lees, Lockett & Roberts (1964). Each collection followed a 2 h fast and began immediately after the oral administration of water or a saline load equivalent to 2·5% of body weight. Individual metabolism cages were used for collection of all urine entering the bladder in the subsequent hour. This period was extended to 2 h for adrenalectomized animals.

*Determinations of time-effect and dose-effect curves.* Cross-over tests made on groups of 16 to 24 rats were used to determine time-effect and then dose-effect curves. *Time-effect curves* were determined by administration of a fixed dose of drug or of vehicle with, or 1, 2, 3, 4 or 6 h before a 1 h collection of urine. *Dose-effect curves* were determined by administration of selected doses of drugs at times fixed for each drug to ensure that the subsequent collections of urine were made during maximum natriuresis. Hence, the collection of urine began immediately after administration of amiloride, 1 h after frusemide and 2 h after either spironolactone or hydrochlorothiazide. Deliberate randomization was used to ensure that all animals received each treatment and that equal numbers of each treatment were administered on each day.

*Measurements of rates of adaptation to diuretic agents.* The dose of diuretics selected produced maximal intensities of natiuresis. Spironolactone (G. D. Searle & Co.) was administered at 9 a.m. and 9 p.m. for 9 days, 1 mg in 0·05 ml propylene

glycol i.m. frusemide (Aust. Hoechst Ltd.) and hydrochlorothiazide (CIBA) were suspended with 0·5% sodium carboxymethylcellulose 40 and 2 mg/ml respectively, and were administered orally at 9 a.m., 4 p.m. and 11 p.m. for 8 days, 1 ml per rat per dose: control animals received 1 ml vehicle. Amiloride (Merck, Sharp & Dohme, Aust.) was given orally to 31 rats for 9 days at 9 a.m., 4 p.m. and 10 p.m., 0·5 mg per rat per dose Eleven of these animals received supplements of 4 mequiv NaCl, 9 received supplements of 4 mequiv KCl per day, each administered orally in 3 divided doses, with the diuretic. Control animals received corresponding supplements or no supplements in water. Urine collections began immediately after administration of the 9 a.m. dose of amiloride, 1 h after the 9 a.m. doses of frusemide, and 2 h after the 9 a.m. doses of hydrochlorothiazide and spironolactone.

*Biochemical methods.* Concentrations of sodium ($Na^+$) and potassium ($K^+$) in trichloracetic acid filtrates of plasma and in urine were determined by flame photometry and were expressed as mequiv/litre and as $\mu$equiv/100 g weight per h, respectively. Concentrations of corticosterone in blood was determined as according to Ilett (1969).

*Measurement of corticosteroidogenesis* in vitro. Both adrenals, removed from rats killed by decapitation, were quartered for incubation as a group in 2 ml Krebs-Hensleit Ringer solution (NaCl, 6·9; KCl, 0·354; $CaCl_2$, 0·282; $NaHCO_3$, 2·1; $KH_2PO_4$, 0·162; $MgSO_4.7H_2O$, 0·294; glucose 2·0 g/litre) at 37° for 2 h, agitating 100 strokes/min under 5% carbon dioxide in oxygen. Corticoids were extracted from the incubation medium as from blood, were separated by thin-layer chromatography and were quantitated (Ilett, 1969).

*Post mortem examinations.* Anaesthesia was induced by 3·6 mg pentobarbitone/100 g rat (i.p.). Blood (4–5 ml) was withdrawn from the abdominal aorta into a heparinized syringe for determination of the haematocrit, plasma electrolytes and blood corticosterone. The spleen, both kidneys and both adrenals were cleaned of adherent fat and weighed. The adrenal glands and alternate left and right kidneys were subjected to routine histological examination. Tissues, fixed in Bouin & Smith's (kidneys) fluid were stained with haematoxylin and eosin. *Fat* was stained in frozen 10 $\mu$m sections of formol-fixed adrenal glands in Oil Red O, counterstaining with haematoxylin. *Juxtaglomerular granules* were stained (Smith, 1966), for evaluation of the juxtaglomerular index (JGI) (Hartroft & Hartroft, 1953). Adrenal and zonal areas were determined by planimetry of traced images (approximately 10 cm in diameter) of stained adrenal sections viewed on the screen of a microscope projection attachment. Serial sections 40 $\mu$m apart were examined; that of largest area, the one preceding and the one following were retained for detailed study. Mean zonal areas for the three sections were calculated. The area of a stage micrometer (1 $mm^2$) projected and measured in the same manner, permitted expression of adrenal areas in $mm^2$.

*Treatment of data.* Data obtained in each experiment were submitted to variance analysis. The significance of differences between means was then determined by *t*-test, within or between groups, as applicable.

## RESULTS

### *Time-effect and dose-effect curves*

Curves relating intensity of natriuresis caused by amiloride, 0·5 mg/rat, hydrochlorothiazide 1·0 mg/rat and frusemide 16 mg/rat to time are shown in Fig. 1. Maximum rates of natriuresis were observed in the first hour after administration of

amiloride and in the third hour after hydrochlorothiazide and frusemide. Hydrochlorothiazide and frusemide had prolonged action; the effect of amiloride was, however, of short duration in the rat. Curves relating intensity of the natriuretic effects of amiloride, hydrochlorothiazide and frusemide to dose are also shown in Fig. 1. Maximum intensities of natriuresis were produced by oral doses of 0·5 mg amiloride, 1 mg hydrochlorothiazide and 40 mg frusemide to rats of 160–194 g.

*Adaptation by rats to the natriuretic actions of various diuretics administered continuously for seven or eight days*

*Frusemide.* The natriuresis and lesser kaliuresis caused by frusemide on the third, fifth and seventh days of continuous treatment, although one half those on the first day, were still four times greater than the control values (Fig. 2). A significant increase in the rate of excretion of water was observed only on initial exposure to the drug.

*Spironolactone* caused a small decrease in the rate of $K^+$-excretion and hence a significant increase in the $Na^+ : K^+$ ratio in the urine which was maintained throughout treatment. The urine volume underwent a small but significant reduction (Fig. 2).

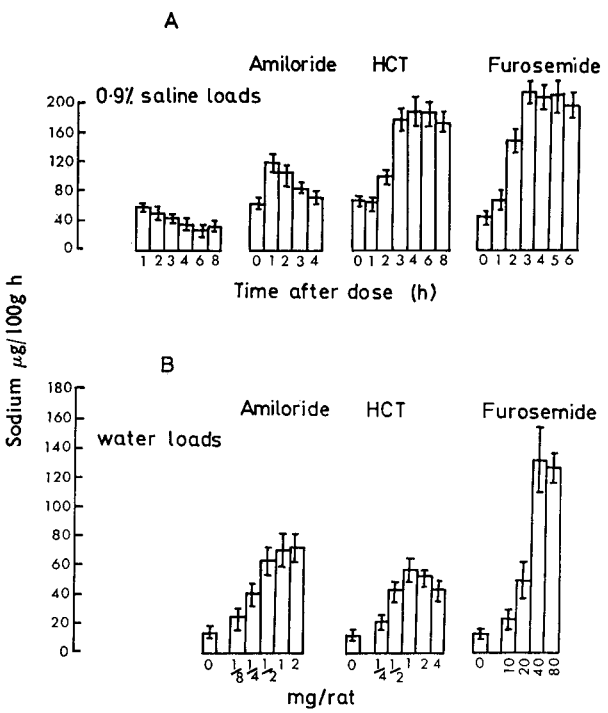


FIG. 1. Curves relating the natriuretic actions of amiloride 0·5 mg/rat, hydrochlorothiazide (HCT) 1·0 mg/rat and frusemide 16 mg/rat to time (top) were obtained from groups of 15, 21 and 21 rats weighing 170 ± 4·1 g, 168 ± 4·6 g and 174 ± 4·8 g, respectively. Individual urine samples were collected throughout the first hour after an oral saline load equivalent to 2·5% body weight, administered at varying time intervals after the oral dose of drug. Curves relating maximum intensity of natriuretic action to dose (bottom) were determined on these same rats. Each animal received an oral water load equivalent to 2·5% body weight either containing a dose of amiloride or 2 h after a dose of HCT or frusemide. Individual urine samples were collected throughout the hour following the fluid load. Both groups of experiments were designed as multiple cross-over tests.

*Amiloride.* In the absence of any supplement, treatment with amiloride (Fig. 2) produced a highly significant diuresis and natriuresis on the first day of treatment. The urinary outputs of $Na^+$ had, however, fallen significantly below control values by the third and had returned to normal by the fifth day and were still normal on the eighth day. Urinary $K^+$ was significantly reduced throughout by amiloride. The provision of a supplement of NaCl reduced the diuretic and enhanced the natriuretic effect of amiloride on the first day of treatment. Normal $Na^+$ excretion had been restored by the third day. Potassium sparing was again a prominent feature of the action of this drug. The provision of a KCl supplement enhanced the diuretic and natriuretic action of amiloride on the first day of exposure to the diuretic, but subsequently the $Na^+$-excretion did not differ from control values. The rate of the urinary excretion of $K^+$ significantly exceeded control outputs only on the fifth day of treatment.

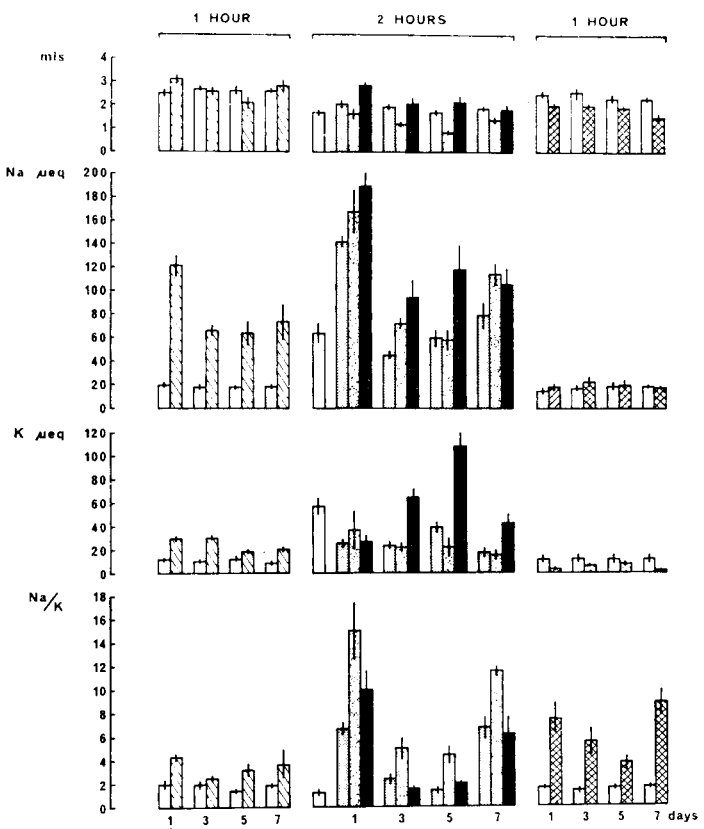


FIG. 2. The excretion of water and electrolytes by rats during eight days of continuous treatment with frusemide (hatched columns), amiloride (stippled columns) or spironolactone (cross-hatched columns). All rats received a water load equivalent to 2·5% body weight at the start of a urine collection of 1 h (frusemide and spironolactone) or 2 h (amiloride). Ordinates: The heights of columns indicate mean values and the inset bars the standard errors of the means; values per 100 g body weight per h. For supplements, given orally with the diuretic, see text. Open columns: control; coarse stippling: amiloride–NaCl (suppl.); solid columns: amiloride–KCl (suppl.).

*Hydrochlorothiazide* administered thrice daily to normal water drinking rats for 8 days, increased the excretion of $Na^+$ significantly on the first and third days of treatment but normal rates of $Na^+$-excretion had been restored by the fifth day (Fig. 3). $K^+$-excretion rose and was maintained at a significantly high level from the third to the eighth and final day of the experiment. The only significant change in urine volume was an increase observed on the first day. Adaptation to the natriuretic action of hydrochlorothiazide did not occur in normal and in adrenalectomized rats provided with NaCl in the drinking fluid (Fig. 3) and was absent also in adrenalectomized animals drinking water and maintained by injection of doca. The kaliuresis caused by hydrochlorothiazide was well sustained in the normal animals drinking saline but was evidenced only intermittently by doca-treated adrenalectomized rats (Fig. 4). The urinary changes caused by hydrochlorothiazide in salt-maintained adrenalectomized rats were markedly subnormal: a significant natriuresis developed only on the eighth day of treatment and kaliuresis was insignificant (Fig. 4).

No significant variations in water and electrolyte excretion occurred from day to day amongst groups of untreated animals examined in parallel with the treated groups.

*Effects of continuous treatment with various diuretic agents on organ weights, plasma electrolytes and the haematocrit value*

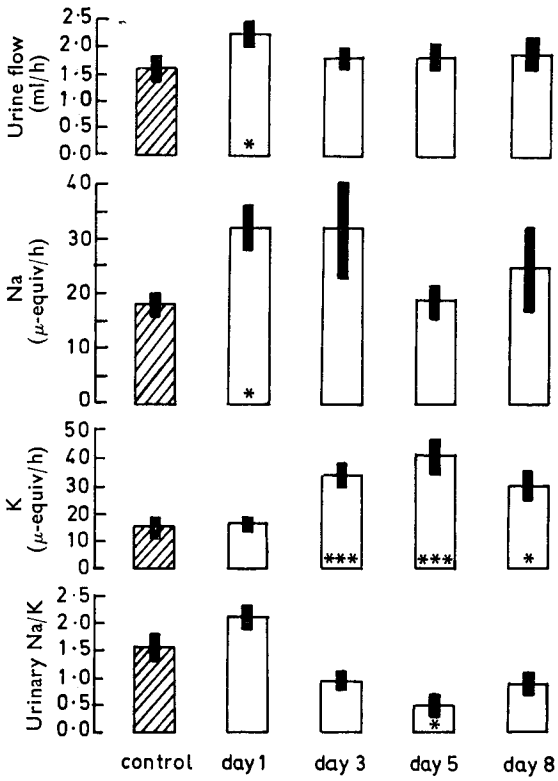


FIG. 3. The excretion of water and electrolytes by normal water-drinking rats during eight days of continuous treatment with hydrochlorothiazide. Measurements of urine volumes and the rates of excretion of $Na^+$ and $K^+$ were made before (shaded columns) and on the first, third, fifth and eighth day of treatment from 11 a.m. to noon, immediately after the administration of a standard water load. The a.m. oral dose of diuretic or of vehicle was administered at 9 a.m. Ordinates as in Fig. 1.

The weights of the adrenals, spleens and thymus glands removed from rats at the termination of nine days of treatment either with hydrochlorothiazide or with spironolactone did not differ significantly from those of corresponding organs removed from control animals; the body weights of the animals, the haematocrit values and plasma electrolytes were also unaffected by these two treatments. In contrast, nine days of treatment with frusemide reduced body weight, the weights of the spleens and thymus glands; the weights of the adrenals were increased (Table 1). Nine days of treatment with amiloride, whether supplemented or unsupplemented, caused a very highly significant reduction in the weight of the thymus and an overall significant ($P < 0{\cdot}05$) decrease in the weight of the spleen. There was no accompanying adrenal hypertrophy or change in body weight. Adrenal weight decreased only in those animals which received a supplement of sodium chloride.

Plasma sodium concentration was reduced from the normal value of $144{\cdot}0 \pm 1{\cdot}83$ (mean $\pm$ the standard error of the mean) mequiv/litre obtained from 40 animals to $132{\cdot}69 \pm 3{\cdot}21$ (9 rats) and $134{\cdot}30 \pm 3{\cdot}36$ (10 rats) by treatment with frusemide and amiloride, respectively. Plasma potassium was reduced from the control value of $4{\cdot}27 \pm 0{\cdot}07$ (40) mequiv/litre to $3{\cdot}90 \pm 0{\cdot}13$ (9) by frusemide and was raised to $4{\cdot}47 \pm 0{\cdot}12$ (11) by amiloride. The haematocrit value was raised from $37{\cdot}66 \pm 0{\cdot}35$ (40) to $43{\cdot}84 \pm 0{\cdot}80$ (9) by frusemide but was unaffected by amiloride.

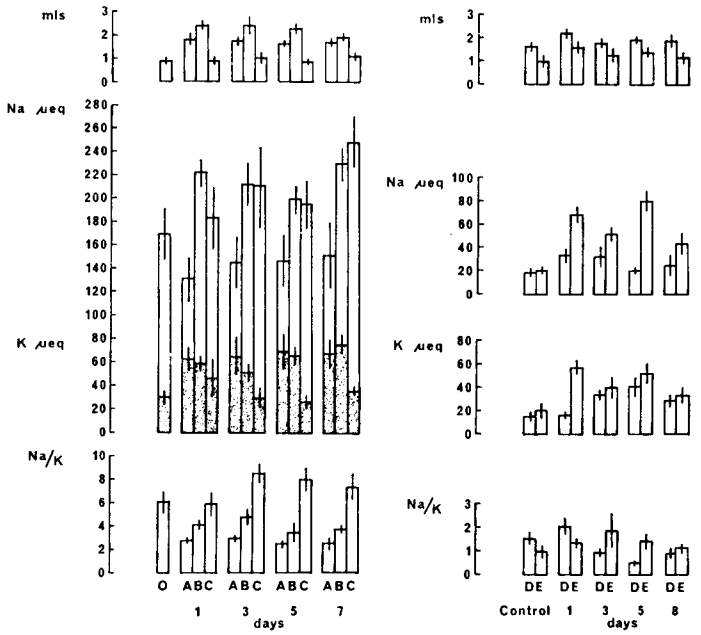


FIG. 4. The excretion of water and electrolytes by rats during eight days of continuous treatment with hydrochlorothiazide. All rats received a load of their drinking fluid equivalent to 2·5% body weight at the start of a urine collection of 1 h duration for intact animals (A, B and D) or of 2 h duration for adrenalectomized rats (C, O and E). Groups D and E drank water; O and C drank 0·9% NaCl; A and B drank 0·7% NaCl. Groups B, C and E received 1 mg hydrochlorothiazide thrice daily: the adrenalectomized rats of Group E were maintained by i.m. injection of doca (250 μg/100 g body wt/day). Ordinates as in Fig. 1.

Table 1. *Effects on organ weights of continuous treatment for nine days with various diuretic agents.*

| | Control | Frusemide | Amiloride | Amiloride + NaCl supplement | Control + KCl supplement | Amiloride + KCl supplement |
|---|---|---|---|---|---|---|
| Body wt (g) .. | 184·50 ± 1·81 (61) | 168·01 ± 4·09 (14)** | 192·55 ± 6·19 (18) | 193·42 ± 8·19 (13) | 176·18 ± 3·62 (14) | 183·47 ± 6·03 (14) |
| Thymus wt (mg) | 182·04 ± 4·46 (61) | 63·04 ± 7·92 (14)*** | 86·15 ± 11·24 (16)*** | 86·15 ± 8·56 (11)*** | 154·61 ± 11·86 (14) | 72·32 ± 12·98 (12)*** |
| Spleen wt (mg) .. | 608·66 ± 13·33 (61) | 333·92 ± 25·44 (14)*** | 538·66 ± 31·79 (18) | 548·79 ± 37·86 (13) | 653·08 ± 35·11 (14) | 515·37 ± 40·61 (14)* |
| Paired adrenal wt (mg) | 30·48 ± 0·45 (61) | 34·44 ± 0·86 (14)*** | 30·72 ± 1·17 (18) | 25·88 ± 1·30 (13)** | 30·08 ± 1·00 (14) | 28·45 ± 1·46 (14) |

The values shown are mean ± their standard errors. Numbers of animals contributing to these means are shown within brackets. Means of *all* control observations on normal rats are shown in column 2. The significance of differences between means obtained from each experimental group and means obtained for the corresponding group of normal control animals has been determined by *t*-test and is indicated by asterisks: one, $P < 0.05$; two, $P < 0.01$; three, $P < 0.001$.

*Effects of continuous treatment with various diuretic agents on the adrenal glands, plasma corticosterone and the juxtaglomerular index*

Continuous treatment with frusemide for nine days increased the maximum area of stained sections of the adrenal glands (Table 2). This increase was due to hypertrophy of zona fasciculata and zona reticularis. In contrast, amiloride given without a supplement, caused enlargement of zona glomerulosa which was offset by reduction in the other cortical zones, particularly zona reticularis, so that the overall adrenal area remained unchanged. A KCl-supplement did not exacerbate these effects of amiloride and the KCl-supplement itself produced no significant changes in zonal areas. The typical effects of amiloride were also presented by animals which had received an NaCl supplement during therapy. This supplement enhanced the extent of the reduction of zona fasciculata induced by amiloride and thereby caused overall reduction in the maximum area of the adrenal cross-sections. The parameters shown in Table 2 were unaffected by treatment either with hydrochlorothiazide or with spironolactone, with the exception of the juxtaglomerular index which was increased from 10·34 to 18·51 by hydrochlorothiazide ($P < 0.001$).

The fat deposits in the zona glomerulosa of the adrenal were markedly increased by treatment with either furosemide or with amiloride. Slight but definite increase in these deposits was also evidenced by animals treated with hydrochlorothiazide. Glands from rats which had received the latter drug synthesized aldosterone *in vitro* at

Table 2. *Effects on adrenal glands, plasma corticosterone and the juxtaglomerular apparatus of continuous treatment for nine days with various diuretic agents.*

| | Control | Furosemide | Amiloride | Amiloride + NaCl supplement | KCl supplement | Amiloride + KCl supplement |
|---|---|---|---|---|---|---|
| Adrenal Glands Total Area (sq mm) | 7·69 ± 0·20 (38) | 9·12 ± 0·32 (12)*** | 7·11 ± 0·30 (8) | 6·63 ± 0·23 (8)** | 6·92 ± 0·29 (8) | 7·49 ± 0·39 (8) |
| Glomerulosa (sq mm) | 0·89 ± 0·02 (38) | 0·88 ± 0·03 (12) | 1·20 ± 0·05 (8)** | 1·20 ± 0·05 (8)** | 0·80 ± 0·04 (8) | 1·11 ± 0·05 (8)** |
| Fasciculata (sq mm) | 4·11 ± 0·12 (39) | 4·88 ± 0·20 (12)** | 3·66 ± 0·18 (8) | 3·27 ± 0·16 (8)* | 3·61 ± 0·11 (8) | 3·63 ● 0·17 (8) |
| Reticularis (sq mm) | 1·50 ± 0·08 (38) | 1·99 ± 0·14 (12)** | 1·04 ± 0·10 (8)** | 1·14 ± 0·13 (8)* | 1·23 ± 0·05 (8) | 1·27 ± 0·12 (8) |
| Corticosterone (μg/100 ml blood) | 15·37 ± 0·67 (40) | 21·25 ± 1·81 (12)** | 9·69 ± 1·58 (9)** | 7·83 ± 1·20 (5)*** | 20·06 ± 2·20 (6) | 13·32 ± 2·14 (7)* |
| Juxtaglomercular Index (%) | 12·27 ± 1·69 (39) | 20·52 ± 1·29 (13)*** | 23·47 ± 1·56 (6)*** | 24·79 ± 2·13 (6)*** | 16·94 ± 1·80 (7) | 25·92 ± 2·15 (7)** |

Explanatory footnote as for Table 1.

a rate of 0·85 ± 0·06 (7) μg/100 mg tissue per 2 h. This rate very significantly exceeded ($P < 0·01$) the corresponding control value of 0·55 ± 0·07 (7). The synthesis of corticosterone was unaffected.

Plasma corticosterone was raised by treatment with frusemide and was lowered by treatment with amiloride. Both treatments raised the juxtaglomerular index (Table 2).

## DISCUSSION

The natriuretic response of normal rats to maximally effective doses of hydrochlorothiazide disappeared by the third day, but the kaliuretic action persisted throughout the eight days of treatment. At the end of this period the haematocrit and the plasma concentrations of $Na^+$ and $K^+$ were within the normal range, but the juxtaglomerular index was significantly raised. This observation confirms a previous report (Tobian, Janecek & others, 1962), is presumptive evidence of an increase in the rate of the synthesis and release of renin (Tobian, 1960) and explains the increased deposits of lipid found in the zona glomerulosa of these glands for increase in these deposits denotes hyperactivity (Eisenstein & Hartroft, 1957; Hartroft & Eisenstein, 1957; Marx & Deane, 1963). Hyperactivity of the z. glomerulosa has been confirmed by demonstration of a significantly raised rate *in vitro* synthesis of aldosterone by adrenals taken from these animals, for aldosterone is synthesized in the rat solely by the cells of this zone (Samuels & Uchikawa, 1967). Since the synthesis of aldosterone is enhanced by angiotensin (Kaplan, 1965) formed by interaction of renin with its plasma protein substrate (Peart, 1965) adaptation to the natriuretic effect of hydrochlorothiazide is attributable to increased activity in the renin-angiotensin-adrenal system. The prolongation of kaliuresis is then attributable to the raised rate of secretion of aldosterone. Experimental support has been presented in substantiation of this hypothesis. First, adaptation to the natriuretic effects of hydrochlorothiazide did not occur in animals on a high salt intake: a high salt diet depresses juxtaglomerular activity (Tobian, 1960), plasma renin activity (Ganong, Biglieri & Mulrow, 1966), the rate of formation of angiotensin and hence the secretion of aldosterone (Müller, 1968). Secondly, adaptation to the natriuretic action of hydrochlorothiazide was prevented by adrenalectomy.

Failure of the NaCl supplement markedly to delay adaptation to amiloride was probably due to the administration of the supplement *with* the drug; the supplement was immediately excreted.

The partial adaptation to the natriuretic but not to the kaliuretic effects of frusemide by the third day of treatment, accompanied by a rise in the juxtaglomerular index, enlargement and increase in the lipid content of the z. glomerulosa, is also attributable to activation of the renin-angiotensin system. The residual natriuresis and kaliuresis in response to frusemide caused loss of body weight, haemoconcentration and reduction in plasma concentrations of $Na^+$ and $K^+$, and appeared to have stressed the animals and hence to have enhanced the secretion of adrenocorticotrophin, with a consequent rise in the concentration of circulating corticosterone. Significant increases in the areas of the zona reticularis and fasciculata of adrenals from frusemide-treated rats accorded, for these zones are known to elaborate corticosterone (Lucis, Carballeira & Venning, 1965; Sheppard, Swenson & Mowles, 1963). The reductions in the weight of the spleen and the involution of the thymus observed in these rats may be attributed to the raised blood level of corticosterone.

The rates of the renal excretion of $Na^+$ had returned to control levels by the third day of continuous treatment with amiloride. On the eighth day, the juxtaglomerular index was raised, the fat deposits in the zona glomerulosa of the adrenal were increased, and the plasma values of corticosterone were subnormal. No explanation of the low levels of corticosterone found can yet be advanced.

In each case, adaptation to the natriuretic actions of hydrochlorothiazide, frusemide and amiloride appears to have been primarily effected by the renin-angiotensin-aldosterone system. Since amiloride exerts its action at a site in the nephron distal to the macula densa (Bull & Laragh, 1968; Crabbé, 1968; Glitzer & Steelman, 1966; Baer, Jones & others, 1967) it appears probable that it is the extrarenal mechanisms (Vander, 1967) and not the intrarenal mechanism (Thurau, 1966) for the control of renin release which are important for the initiation and maintenance of adaptation to natriuretic action. This adaptive process extends over days and appears distinct from the acute effects of natriuretic agents on renin secretion (Cooke, Brown & others, 1970; Meyer, Menard & others, 1968) which appear to be of intrarenal origin. The minor significance of the renin-angiotensin system for the control of aldosterone secretion under normal circumstances (Palmore & Mulrow, 1967) and after nephrectomy (Palmore, Marieb & Mulrow, 1969) does not preclude the possible importance of this system for adaptation to natriuretic actions of drugs in this species. Moreover Kinson & Singer (1968) have clearly demonstrated that angiotensin increases the secretion of aldosterone in the rat during negative sodium balance.

The component of the overall actions common to the effects of these diuretics was salt loss and salt loss was contrastingly accompanied by dehydration (frusemide), $K^+$ loss (frusemide and hydrochlorothiazide) and $K^+$ retention (amiloride) potassium loss without salt loss caused no adaptation (spironolactone). The mechanism by which salt loss provokes the onset of the positive $Na^+$-balance which restores extracellular fluid volume without suppressing the activity of the renin-angiotensin-aldosterone system still requires clarification. Recently, frusemide and hydrochlorothiazide have been shown to increase the rate of excretion of catecholamines in the urine (Heidland & Heinemann, 1969) and excitation of the renal sympathetic nerves enhances the secretion of renin (Vander, 1967). It is, therefore, possible that activity in the sympathetic nervous system may contribute to the slow adaptive process to natriuretic agents.

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