*J. Pharm. Pharmacol.*, 1965, **17**, 368–375 Received January 13, 1965

# Diuretic effect of some adrenocortical steroids in the rat

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Cortisone, hydrocortisone and some of their $\Delta^1$- and fluorinated analogues have a pronounced diuretic effect in the rat; this is accompanied by increased urinary excretion of both $Na^+$ and $K^+$. Diuresis is maximal some 4 hr after oral administration, and in acute studies, the diuretic potency of some of the newer corticosteroids exceeds 100 times that of chlorothiazide.

PROLONGED administration of some adrenocortical steroids to rats causes retardation of body growth (D'Arcy & Howard, 1958a, b, 1961a, 1962). Although it was certain that this effect was due to the action of these steroids on protein catabolism and in particular on the formation of carbohydrate from protein, it was observed that urine excretion was increased, and it was thought that tissue dehydration might be an influencing factor in the growth retardation.

Fielder, Hoff, Thomas, Tolksdorf, Perlman & Cronin (1959), and Wozniak, Paino, Ringler & Roepke (1960) have shown that triamcinolone, administered acutely or sub-acutely to dogs, produces a diuresis and a significant loss of body weight. Several clinicians (Bilka & Melby, 1958; Curd & Spurr, 1958; Feinberg, Feinberg & Fisherman, 1958; Freyberg, Berntsen & Hellman, 1958) have also observed natriuresis and diuresis among the untoward effects following the use of some newer corticosteroids in man.

We have assessed the effect of acute and sub-acute doses of various corticoids on urinary output in the rat and have determined whether this influenced the loss of body weight.

## Experimental

Male albino rats, 120–150 g weight, of the Tuck strain were used. They were housed in a thermostatically controlled room at 68–70° F and maintained on a cube diet; tap water was provided *ad lib.* Adrenocortical steroids and chlorothiazide were administered orally in a dose volume of 0·5 ml/100 g weight, to groups of 4 or 5 rats. Control groups were given the diluent alone (5% gum acacia in distilled water) in similar dose volumes. Deoxycortone acetate and a long-acting preparation of adrenocorticotrophic hormone (Cortrophin-ZN) were injected intramuscularly, half of each dose into each thigh muscle; deoxycortone acetate was dissolved in arachis oil and Cortrophin-ZN diluted in saline. Controls were injected with similar volumes of arachis oil or saline intramuscularly.

Urine was collected according to Brittain (1959), and urine excretion was measured over a period of 8 hr during which the animals were deprived of both food and water. Rats were conditioned to the urine collection apparatus before their use in diuretic tests (D'Arcy, 1962). The levels of $Na^+$ and $K^+$in the urine were estimated using a flame photometer.

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## Results

### EFFECT OF CORTICOSTEROIDS ON URINE OUTPUT

The oral administration of cortisone acetate and hydrocortisone acetate (0·63, 1·25, 2·5 and 10 mg/100 g), prednisone (0·31, 0·63 and 2·5 mg/100 g) and prednisolone acetate (0·16, 0·31, 0·63, 1·25 and 2·5 mg/100 g) produced a demonstrable increase in urine output. Urine excretion was maximal some 4 hr after dosage for the steroids examined; Fig. 1 illustrates the diuretic effect of cortisone acetate.

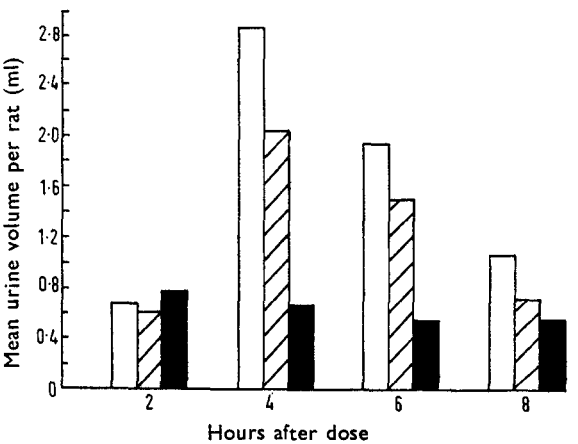


FIG. 1. Mean urine excretion per rat at 2 hr intervals after oral dosage with cortisone acetate. Each dose of the steroid was given in a volume of 0·5 ml/100 g body weight to 2 groups of 4 rats; control rats received a similar dose of the vehicle. Open columns: cortisone acetate 10 mg/100 g. Hatched columns: cortisone acetate 2·5 mg/100 g. Solid columns: controls.

The diuretic effect of these steroids increased with increase in dosage; in Fig. 2 the degree of diuresis has been expressed by a "Diuretic Index", which is the ratio of urine volume from treated animals to that of the controls. Chlorothiazide (0·63–20 mg/100 g) was included for comparison.

All these steroids showed greater diuretic activity than chlorothiazide in total urine excretion for 8 hr after dosage. 9α-Fluorohydrocortisone (fludrocortisone) acetate (0·31–5·0 mg/100 g), $\triangle^1$-9α-fluorohydrocortisone (0·16–1·25 mg/100 g) and 9α-fluoro-16α-hydroxyprednisolone (triamcinolone) (0·02–1·25 mg/100 g) had an even greater effect on urine excretion. Deoxycortone acetate (1·0–25 mg/100 g) produced some diuresis but the effect was much less than that produced by chlorothiazide or by any of the other steroids; similarly Cortrophin-ZN had diuretic activity when injected intramuscularly at doses of 1·25–10 mg/100 g, although it was less potent than chlorothiazide. This response, although weak, indicates that stimulation of the output of endogenous adrenal steroids also promotes diuresis. These results are summarised in Table 1, and an approximation of the diuretic potency of the steroids relative to chlorothiazide under the conditions of the test has been made in Table 2.

TABLE 1. THE DIURETIC EFFECT OF SOME ADRENOCORTICAL STEROIDS, CORTICOTROPHIN AND CHLOROTHIAZIDE

| Steroid or other agents | Dose mg/100 g/ orally | No. of rats | Mean urine volume per test rat per 8 hr (ml) | Mean urine volume per control rat per 8 hr (ml) | Diuretic index |
|---|---|---|---|---|---|
| Cortisone acetate | 10 | 8 | 6·55 | 2·56 | 2·6 |
| " " | 2·5 | 8 | 4·84 | 2·56 | 1·9 |
| " " | 1·25 | 8 | 3·45 | 1·76 | 2·0 |
| " " | 0·63 | 8 | 3·13 | 1·76 | 1·8 |
| Hydrocortisone acetate | 10 | 5 | 6·98 | 2·49 | 2·8 |
| " " | 2·5 | 5 | 6·26 | 2·49 | 2·5 |
| " " | 1·25 | 10 | 7·57 | 3·35 | 2·3 |
| " " | 0·63 | 10 | 5·06 | 3·35 | 1·5 |
| Prednisone | 2·5 | 4 | 6·33 | 1·90 | 3·3 |
| " | 0·63 | 9 | 4·34 | 1·65 | 2·6 |
| " | 0·31 | 5 | 1·58 | 1·45 | 1·1 |
| " | 0·16 | 5 | 1·30 | 1·45 | 0·9 |
| Prednisolone acetate | 2·5 | 4 | 6·73 | 2·54 | 2·6 |
| " " | 1·25 | 4 | 6·70 | 2·54 | 2·6 |
| " " | 0·63 | 9 | 5·83 | 2·45 | 2·4 |
| " " | 0·31 | 9 | 5·30 | 2·45 | 2·2 |
| " " | 0·16 | 5 | 3·04 | 2·38 | 1·3 |
| " " | 0·08 | 5 | 3·22 | 2·38 | 1·4 |
| Fludrocortisone acetate | 5·0 | 4 | 5·88 | 1·19 | 4·9 |
| " " | 1·25 | 4 | 5·53 | 1·19 | 4·6 |
| " " | 0·63 | 5 | 5·42 | 1·67 | 3·2 |
| " " | 0·31 | 4 | 2·43 | 1·19 | 2·0 |
| " " | 0·16 | 5 | 4·14 | 1·67 | 2·5 |
| $\Delta^1$-9α-Fluorohydrocortisone acetate | 2·5 | 4 | 6·50 | 1·90 | 3·4 |
| " " | 1·25 | 5 | 6·68 | 1·23 | 5·4 |
| " " | 0·63 | 9 | 5·98 | 1·52 | 3·9 |
| " " | 0·31 | 5 | 5·00 | 1·23 | 4·1 |
| " " | 0·16 | 5 | 4·02 | 1·23 | 3·3 |
| Triamcinolone | 1·25 | 5 | 5·67 | 1·56 | 3·6 |
| " | 0·63 | 5 | 5·68 | 1·56 | 3·6 |
| " | 0·31 | 5 | 5·04 | 1·56 | 3·2 |
| " | 0·16 | 5 | 5·15 | 1·35 | 3·8 |
| " | 0·08 | 10 | 4·59 | 1·82 | 2·5 |
| " | 0·04 | 10 | 2·90 | 2·33 | 1·3 |
| " | 0·02 | 10 | 2·86 | 2·33 | 1·2 |
| " | 0·01 | 5 | 2·35 | 2·35 | 1·0 |
| Deoxycortone acetate | 25* | 4 | 2·01 | 1·04 | 1·9 |
| " " | 10* | 8 | 2·81 | 2·04 | 1·4 |
| " " | 1·0* | 4 | 2·62 | 2·90 | 0·9 |
| Cortrophin-ZN | 10* units | 8 | 4·20 | 2·06 | 2·0 |
| " | 5·0* units | 8 | 3·60 | 2·45 | 1·5 |
| " | 2·5* units | 8 | 4·23 | 2·60 | 1·6 |
| " | 1·25* units | 8 | 3·21 | 2·40 | 1·3 |
| Chlorothiazide | 20 | 15 | 3·77 | 1·63 | 2·4 |
| " | 5·0 | 15 | 3·09 | 1·63 | 1·9 |
| " | 2·5 | 10 | 4·37 | 2·39 | 1·8 |
| " | 1·25 | 10 | 3·85 | 2·10 | 1·8 |
| " | 0·63 | 15 | 3·31 | 2·40 | 1·4 |

* Intramuscularly

TABLE 2. APPROXIMATE RELATIVE DIURETIC ACTIVITY OF SOME ADRENOCORTICAL STEROIDS, CORTICOTROPHIN AND CHLOROTHIAZIDE

| Diuretic agent and route | Relative diuretic activity |
|---|---|
| Chlorothiazide (oral) | 1 |
| Cortisone acetate (oral) | 1–2 |
| Hydrocortisone acetate (oral) | 4–8 |
| Prednisone (oral) | 32–64 |
| Prednisolone acetate (oral) | 32–64 |
| Fludrocortisone acetate (oral) | 64 |
| $\Delta^1$-9α-Fluorohydrocortisone acetate (oral) | >128 |
| Triamcinolone (oral) | >128 |
| Deoxycortone acetate (i.m.) | <1 |
| Cortrophin-ZN (i.m.)* | <1 |

* Compared as units of Cortrophin-ZN against mg of chlorothiazide.

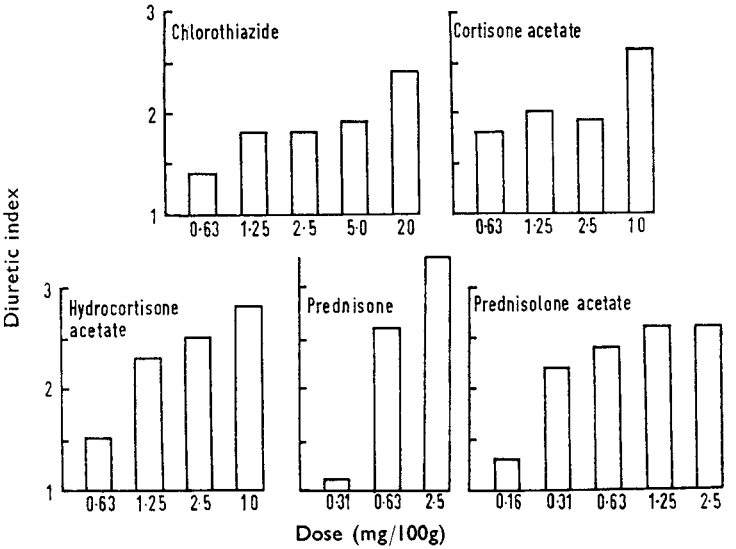


FIG. 2. The effect of oral dosage with chlorothiazide and some corticosteroids on urine excretion in groups of 4 or 5 rats. Urine was collected during 8 hr after dosage. The Diuretic Index is the ratio of urine volume from treated animals to that of the controls.

EFFECT OF CORTICOSTEROIDS ON ELECTROLYTE EXCRETION

The $Na^+$ and $K^+$ levels in the urine were measured routinely. These results (Table 3), relate urine output to the concentration of $Na^+$ and $K^+$ in the urine, and to the total $Na^+$ and $K^+$ excretion per rat during the 8 hr

TABLE 3. THE EFFECT OF ORAL DOSAGE OF SOME ADRENOCORTICAL STEROIDS AND CHLOROTHIAZIDE ON THE $Na^+$ AND $K^+$ LEVELS IN THE URINE OF RATS

| Treatment | Dose mg/100 g | No. of rats | Mean urine volume excreted per rat (ml) during 8 hr | $Na^+$ conc in urine m-equiv./ litre | $K^+$ conc in urine m-equiv./ litre | Total $Na^+$ excreted per rat (m-equiv.) in 8 hr | Total $K^+$ excreted per rat (m-equiv.) during 8 hr |
|---|---|---|---|---|---|---|---|
| Controls .. .. | — | 131 | 2·0 s.e. ± 0·2 | 150·7 s.e. ± 9·8 | 122·5 s.e. ± 8·6 | 0·28 s.e. ± 0·02 | 0·23 s.e. ± 0·01 |
| Hydrocortisone acetate .. .. | 10 | 5 | 7·0 | 130·4 | 76·9 | 0·91 | 0·54 |
| ,, ,, | 2·5 | 5 | 6·3 | 143·5 | 82·1 | 0·90 | 0·52 |
| ,, ,, | 0·63 | 10 | 5·0 | 134·8 | 87·3 | 0·70 | 0·44 |
| Prednisone .. .. | 2·5 | 4 | 6·3 | 104·3 | 66·7 | 0·66 | 0·42 |
| ,, .. .. | 0·63 | 9 | 4·3 | 147·8 | 79·5 | 0·64 | 0·34 |
| ,, .. .. | 0·16 | 5 | 1·3 | 243·4 | 169·3 | 0·32 | 0·22 |
| Fludrocortisone acetate .. | 5·0 | 4 | 5·9 | 95·6 | 110·3 | 0·56 | 0·65 |
| ,, ,, .. | 1·25 | 9 | 5·8 | 89·2 | 116·7 | 0·52 | 0·68 |
| ,, .. | 0·31 | 4 | 2·4 | 95·6 | 148·8 | 0·23 | 0·36 |
| $\Delta^1$-9αFluorohydrocortisone acetate .. | 2·5 | 4 | 6·5 | 143·5 | 59·0 | 0·93 | 0·38 |
| ,, ,, .. | 0·63 | 9 | 6·0 | 141·3 | 116·8 | 0·85 | 0·70 |
| ,, ,, .. | 0·16 | 5 | 4·0 | 130·4 | 153·9 | 0·52 | 0·62 |
| Triamcinolone .. | 1·25 | 10 | 5·7 | 136·9 | 103·9 | 0·78 | 0·59 |
| ,, .. | 0·31 | 10 | 5·0 | 123·9 | 101·4 | 0·62 | 0·51 |
| ,, .. .. | 0·08 | 10 | 5·4 | 108·6 | 69·3 | 0·59 | 0·37 |
| ,, .. .. | 0·02 | 10 | 3·5 | 108·6 | 77·0 | 0·38 | 0·27 |
| Chlorothiazide .. | 20 | 5 | 3·3 | 147·8 | 92·4 | 0·49 | 0·30 |
| ,, .. .. | 5·0 | 5 | 2·3 | 165·2 | 84·7 | 0·38 | 0·20 |
| ,, .. .. | 2·5 | 5 | 2·9 | 165·2 | 82·1 | 0·48 | 0·24 |

test. Control results have been pooled in the Table, and the mean values (± s.e.) calculated; they represent results from 131 rats in 27 groups.

With all steroids, the $Na^+$ concentration in the urine decreased; this did not seem to be directly related to the dose of an individual steroid. There was also a decreased concentration of $K^+$ and this seemed to be related to the dose of the steroid except triamcinolone. When the $Na^+$ and $K^+$ values were expressed as total m-equiv. of electrolyte excreted per rat during 8 hr, the $Na^+$ excretion increased beyond that of the controls and appeared to become greater as the dose of the steroid increased. The $K^+$ excretion increased although not to levels as high as those of $Na^+$; this effect was apparently related to the dose of the steroid. With fludrocortisone, the ratio of $Na^+$ to $K^+$, whether as concentration in the urine or as total electrolyte excreted per rat during 8 hr was less than one; with all the other steroids, this ratio was greater than one.

Chlorothiazide, in the doses used, had little effect on the $Na^+$concentration in the urine, although it did raise the total excretion of this ion during 8 hr. The $K^+$ concentration was slightly lower in the treated than in the control animals although the total amount of $K^+$ excreted per rat during 8 hr was normal.

EFFECT OF PROLONGED DOSAGE ON DIURESIS AND ON BODY WEIGHT

The effect on diuresis of steroid given orally was assessed over 14 days, and was compared with the effect of chlorothiazide. Fludrocortisone was selected since it was one of the more potent steroids producing diuresis.

Three groups of 5 rats were used; one group received daily oral doses of fludrocortisone acetate (1·25 mg/100 g), the second group were given chlorothiazide (2·5 mg/100 g), and a control group received the diluent alone (5% gum acacia in distilled water). All animals received their daily dosage in a volume of 0·5 ml/100 g; food and water were withheld during the 8 hr test but animals had unlimited access to both on the days on which, although dosed, they were not placed in the urine collection apparatus. Urine volumes per group were recorded on the first day of the experiment and subsequently at 2 or 3 day intervals; weights were also recorded at these times. The effect of continued dosage on urine output and on weight is shown respectively in Figs 3 and 4.

Daily administration of each drug produced a diuretic effect, which showed a gradual decrease during the 14 days. This reduction was more apparent with the steroid than with chlorothiazide. Although towards the end of the experiment, chlorothiazide produced a diuresis similar to that of the steroid, the weight of the two treated groups differed. The rats dosed with the steroid did not maintain a normal rate of growth, whereas those dosed with chlorothiazide continued to gain weight and the mean body weight did not differ significantly from that of the control animals.

In earlier studies (D'Arcy, Brittain & Howard, 1961), in which rats were treated with adrenal steroids (prednisone, 0·63 and 2·5 mg/100 g; $\Delta^1$-9$\alpha$-fluorohydrocortisone, 0·63 and 2·5 mg/100 g) orally on alternate days over a period of 14 days, there was no decrease in the diuretic effect.

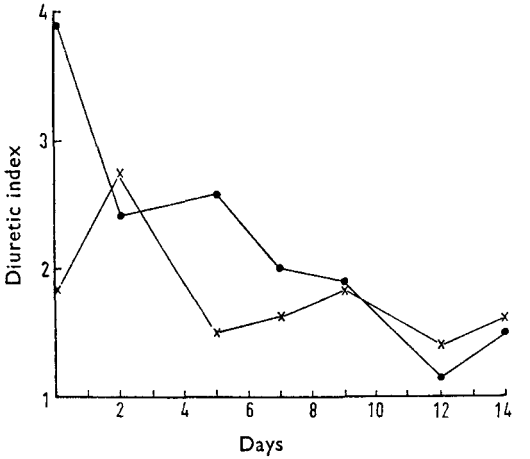


FIG. 3. A comparison between the diuretic effect of chlorothiazide (2·5 mg/100 g orally per day), (×——×) and fludrocortisone acetate (1·25 mg/100 g orally/day), (●——●) during sub-acute (14 days) administration to rats. Groups of 5 rats were used.

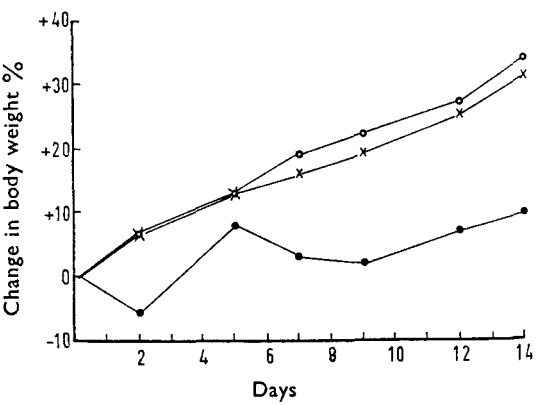


FIG. 4. The effect of daily oral administration of chlorothiazide and fludrocortisone acetate on the body weight of rats. Groups of 5 rats were used; controls (○——○); chlorothiazide (2·5 mg/100 g/day), (×——×); fludrocortisone acetate (1·25 mg/100 g/day), (●——●).

## Discussion

Cortisone, hydrocortisone and some of the newer corticosteroids have a pronounced diuretic effect in the rat. This is maximal some 4 hr after oral administration. Acutely, these adrenocortical steroids were more potent diuretics than chlorothiazide; this potency was especially evident with the $\Delta^1$-and fluorinated steroids where maximal activity was at least 100 times ($>128$) that of chlorothiazide. Cortrophin-ZN, when injected intramuscularly, also had a diuretic effect which, although weak relative to that of the other corticosteroids, suggests that stimulation of the adrenal cortex will also produce diuresis.

Hydrocortisone and its $\Delta^1$- and fluorinated analogues uniformly elicit increases in the urinary excretion of both sodium and potassium; however, the $Na^+$- and $K^+$-retaining properties of these steroids are also evident from a comparison of the amounts of the two electrolytes in the urine with the amounts present in urine from control animals. The increase in water excreted is responsible for the overall loss of electrolytes.

The mechanism of the corticosteroid-induced diuresis is uncertain; Heller & Ginsburg (1961) have suggested that the rise in glomerular filtration rate and renal blood flow, which the glucocorticoids usually produce in healthy animals and man, may be a factor of importance. Alternatively, a direct effect on tubular water reabsorption has been both postulated (Jones, 1957) and denied (Skillern, Corcoran & Scherbel, 1956).

Interaction between the antidiuretic hormone (ADH) and the corticosteroid is yet another possibility, and this theoretically could involve a suppression or release of ADH by the supraopticohypophysial system, or an interaction between ADH and the steroid at a common site of action, notably the renal tubule. Initial experiments reported elsewhere (D'Arcy & Howard, 1961b) add support to the suggestion that corticosteroid-ADH interaction may be one of the mechanisms involved in the diuresis, although the findings were not sufficiently extensive to suggest the possible site of this interaction. Prednisolone reduces the antidiuretic effect of vasopressin (Natzschka & Senft, 1959); some corticosteroids interfere with the release of ADH from the neurohypophysis (Gaunt, Lloyd & Chart, 1957; Martini, Pecile & Giuliani, 1960) and they will also promote the disappearance of ADH from the circulation (Ginsburg, 1954).

Changes in weight are the result of a gain or loss of anhydrous tissue and a gain or loss of water; in earlier studies (D'Arcy & Howard, 1960; D'Arcy, Brittain & Howard, 1961) it was suggested that the retardation of body growth in rats dosed subacutely or chronically with adrenocortical steroids might be due partially to dehydration caused by diuresis. In the present work, after daily administration of chlorothiazide and fludrocortisone to rats for 14 days, there was a gradual decrease in the diuretic effect. This rate of decrease was greater with the steroid, which initially was the more potent. After 14 days the diuretic effect of the two drugs was the same. The rats treated with the chlorothiazide daily had a normal increase in weight, whereas rats treated with fludrocortisone showed retardation of growth. It is thus apparent that the failure of rats to gain weight during prolonged corticosteroid dosage is unlikely to be influenced by the diuresis.

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