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TRIAMTERENE, A NEW DIURETIC DRUG

I. STUDIES IN NORMAL MEN AND IN ADRENALECTOMIZED RATS

BY

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Triamterene (2,4,7-triamino-6-phenylpteridine) represents a new type of diuretic drug. It has been shown to have a diuretic effect in normal subjects (Hild and Krueck, 1961; Laragh *et al.*, 1961; Crosley *et al.*, 1962), producing increased excretion of sodium and water but a decrease in potassium excretion. This effect was at first attributed mainly to aldosterone antagonism (Crosley *et al.*, 1961; Wiebelhaus *et al.*, 1961). Hild and Krueck (1961) suggested that, in addition to aldosterone antagonism, triamterene probably had an independent direct effect on the kidneys.

We have studied the effect of triamterene in normal subjects and in adrenalectomized rats. Our results suggest that the natriuresis and potassium retention are due to direct action on renal tubular cells.

Studies in Normal Men

The observations were made on eight healthy men aged 26-44 years. During the studies the subjects carried out their normal hospital and laboratory duties and a normal diet was taken. When a high salt intake was required 12 g. of sodium chloride daily was added to the normal diet.

The following substances were taken by mouth during the experiments: triamterene (as capsules), hydroflumethiazide and fludrocortisone acetate (both as tablets). The dosage regime is indicated below.

Urine was collected at two-hourly intervals for ten hours during the period 8 a.m. to 6 p.m., after giving the drug at 8 a.m. At least two days were allowed to elapse before a further dose was given. Similar collections were also made on other days when no drugs were given.

Studies in Adrenalectomized Rats

Male albino rats weighing initially 250-300 g. were used. Adrenalectomy was performed under ether anaesthesia, and the rats were kept in a controlled-temperature room (25° C.). They were given a constant diet of commercial rat cake and were allowed to drink 1% sodium chloride solution freely. All experiments were performed at least two weeks after adrenalectomy.

The rats were studied in groups of three in each metabolism cage, and the urine collection from each group was pooled. At the beginning of each experiment the bladder was emptied by gentle suprapubic pressure. An intragastric tube was passed and the required dose of triamterene or spironolactone suspended in 4 ml. of water was introduced into the stomach. Control groups of rats received 4 ml. of water by the same route. Urine collections were made from each group of rats for five hours and then the bladders were emptied again.

Chemical Procedures

The concentrations of sodium and potassium in all urine samples were determined by flame photometer. Chloride was estimated by potentiometric titration with silver nitrate (Sanderson, 1952) and bicarbonate by a microdiffusion method (Conway, 1950). Titratable acidity and pH of urine were measured by a Marconi pH meter.

Measurement of triamterene in urine samples was carried out by fluorimetry, using the Hilger H 730 fluorescent attachment in conjunction with Hilger H 700 Uvispek spectrophotometer. The highest concentration of triamterene that could be measured by the fluorimeter was 0.1 µg./ml. A stock solution of 400 µg./ml. of triamterene was prepared in 98% formic acid. From this, two series of seven dilutions of triamterene ranging from 0.1 µg./ml. to 0.005 µg./ml. were made, the first series by diluting with water and the second series by diluting with water containing 1:2,000 parts normal urine. The concentration of triamterene was plotted against the percentage transmission scale reading of the spectrophotometer, and two parallel straight lines were obtained, the dilutions containing urine giving consistently slightly higher readings. In preparation of urine samples for fluorimetry, 0.1 ml. of urine was made up to 200 ml. with 0.02% formic acid. A blank was prepared from urine passed by the same subject just before triamterene had been given. As a reference standard, triamterene solution 0.1 µg./ml. was used. The spectrophotometer scale was set to 100% transmission with the reference standard, and the blank and the unknown solutions were measured. After subtracting the reading of the blank, which was always less than 3%, the concentration of triamterene in the urine was determined from the calibration curve. To check the reproducibility of the method, the same urine sample was estimated twelve times and the coefficient of variation was 1%.

Results in Normal Men

Response to Increasing Doses of Triamterene

The effect of triamterene in doses of 50-300 mg. was studied in four subjects while on an ordinary diet. All the doses used increased sodium excretion and produced considerable decrease in potassium excretion. The effect of the oral dose was apparent in the first two hours and the maximal effect occurred after four to six hours. In

each case a faint blue colour of the urine was noticed after the ingestion of triamterene.

The detailed estimations from the two-hourly urine collections with each dose of triamterene in one subject on a normal diet are shown in Fig. 1. Similar results were obtained in the other three subjects. The total ten-hourly volumes of urine and excretions of sodium and potassium in the four subjects are summarized in Table I. In four normal subjects, triamterene (200 mg.)

High Sodium Chloride Intake

To the normal diet of four subjects 12 g. of sodium chloride daily was added for five days. Urine was collected at two-hourly intervals for ten hours, on the third, fourth, and fifth days. A single dose of 200 mg. of triamterene was given at 8 a.m. on the fifth day.

Triamterene produced an increase in urine volume and sodium excretion, and a considerable decrease in potassium excretion. The detailed results for one subject are shown in Fig. 4 and similar results were obtained with the other three subjects. There was no consistent difference between the extent of potassium retention when on high sodium intake and on a normal diet (Table II).

Excretion of Triamterene in Urine

A capsule containing 200 mg. of triamterene without excipient was given in four normal subjects at 8 a.m. Urine was collected for 24 hours in the following manner: hourly collections for the first six hours, two-hourly collections for the next six hours, and a single twelve-hour collection. The maximum excretion of the drug occurred during the second and third hours after ingestion

FIG. 1.—Response to increasing doses of triamterene in one normal subject, showing the two-hourly urine excretion of water, sodium, and potassium for 10 hours.

produced considerable increases in the amounts of bicarbonate and chloride excreted, the former being rather larger, particularly during the first four hours. During the same period there was a conspicuous increase in urinary pH. The results in one subject are shown in Fig. 2, and similar results were obtained in each case.

Effect of Fludrocortisone

Fludrocortisone acetate was given to two normal subjects on an ordinary diet, 1 mg. at 12 midnight and 1.5 mg. at 8 a.m. Similar results were obtained in both subjects (Fig. 3). Sodium excretion was reduced and the potassium excretion increased after fludrocortisone. Hydroflumethiazide given in addition at 8 a.m. led to natriuresis and the potassium loss was increased still further. Triamterene, however, reversed the action of fludrocortisone and considerably reduced the potassium excretion, particularly during the first four hours.

(Fig. 5). The total excretion in 24 hours was 15–30% of the oral dose.

Results in Adrenalectomized Rats

Triamterene in the range of 0.5–32 mg. and spironolactone 2–16 mg. were given, as single doses by intragastric tube, to the adrenalectomized rats.

The doses of 1 mg. of triamterene and 2 mg. of spironolactone (in the rat) are equivalent on a weight basis to 200 mg. of triamterene and 400 mg. of spironolactone respectively in a man of 70 kg. (triamterene 3 mg./kg. and spironolactone 6 mg./kg. body weight approximately).

Triamterene enhanced the excretion of sodium and produced a considerable decrease in potassium excretion. This effect was apparent with the smallest dose used (0.5 mg./rat) and increased with the increasing dosage (Fig. 6). In contrast spironolactone produced no

TABLE I.—Urinary Excretion of Sodium and Potassium During 10 Hours in Four Normal Men, Showing Effect of Increasing Doses of Triamterene

Subject	Age (yr.)	Weight (kg.)	No. of Observations	Control			Triamterene 50 mg.			Triamterene 100 mg.			Triamterene 200 mg.			Triamterene 300 mg.		
				Volume (ml.) Mean $\pm$ S.D.	Sodium mEq Mean $\pm$ S.D.	Potassium mEq Mean $\pm$ S.D.	Volume (ml.)	Sodium (mEq)	Potassium (mEq)	Volume (ml.)	Sodium (mEq)	Potassium (mEq)	Volume (ml.)	Sodium (mEq)	Potassium (mEq)	Volume (ml.)	Sodium (mEq)	Potassium (mEq)
I	26	74	7	481 $\pm$ 37	75.5 $\pm$ 13.9	44.3 $\pm$ 10.2	510	126	24	560	128	34	670	150	28	970	208	21
II	30	74	5	801 $\pm$ 396	80.0 $\pm$ 31.0	38.0 $\pm$ 4.8	575	116	34	940	157	27	1,071	120	19	1,232	168	13
III	38	77	4	774 $\pm$ 153	98.4 $\pm$ 21.2	56.3 $\pm$ 9.9	840	131	36	1,035	159	24	1,026	164	20	2,270	135	22
IV	44	78	4	765 $\pm$ 214	57.3 $\pm$ 15.3	49.4 $\pm$ 9.2	940	95	35	820	88	26	1,200	134	39	1,235	154	32

appreciable change in sodium or potassium excretion in the adrenalectomized rats (Table III).

Discussion

Triamterene produced an increase in sodium, chloride, and bicarbonate excretion and a considerable decrease in potassium excretion in normal subjects. The natriuretic effect of triamterene in normal subjects, when given an ordinary diet, a high salt diet, or when under the influence of fludrocortisone was less than that of hydroflumethiazide. Urinary bicarbonate was increased after administration of triamterene. However, the natriuresis produced by triamterene does not resemble that due to a carbonic anhydrase inhibitor, as with the latter there is usually an associated increase in potassium excretion (Counihan *et al.*, 1954; Pitts, 1958).

The decrease in potassium excretion produced by triamterene in the normal subjects given an ordinary diet is difficult to explain on the basis of aldosterone antagonism. In health the level of endogenous aldosterone secretion is low (Axelrad and Luetscher, 1954), and it has been found that spironolactone has little effect on electrolyte excretion in normal men receiving an ordinary diet (Edmonds and Wilson, 1960). Triamterene depressed potassium excretion in normal subjects given additional sodium, which reduces the level of endogenous aldosterone to a minimum (Mulrow *et al.*, 1956; Ulick *et al.*, 1958). In normal subjects the potassium retention resulting from triamterene was as

great when a high sodium intake was given as with an ordinary diet; this is further evidence that the potassium-conserving action of triamterene does not depend mainly on aldosterone antagonism.

FIG. 2.—Effect of triamterene on the urinary pH and on sodium, potassium, chloride, and bicarbonate excretion in one normal subject on an ordinary diet. Observations refer to two-hourly collections of urine.

FIG. 3.—Influence of triamterene and hydroflumethiazide on the effect of fludrocortisone on urine volume and on sodium and potassium excretion in one normal subject.

FIG. 4.—Effect of triamterene on the urine volume and on sodium and potassium excretion in one normal subject who was receiving a high sodium-chloride intake.

Triamterene, like spironolactone, when given with hydrocortisone reverses the effects of the latter on sodium and potassium excretion. It does not necessarily follow that its mode of action is due to competitive inhibition of the mineralo-corticoids. Similar results would be obtained if triamterene had a direct effect on the renal tubules.

TABLE II.—Potassium Excretion After Administration of Triamterene to Healthy Subjects Receiving Normal and High Sodium Diets

Subject	Normal Diet				Normal Diet with Addition of 12 g. NaCl Daily			
	K Excretion mEq/10 Hours		Decrease in K Excretion		K Excretion mEq/10 Hours		Decrease in K Excretion	
	Control (Mean)*	Triamterene 200 mg.	mEq/10 Hours	% of Control	Control†	Triamterene 200 mg.	mEq/10 Hours	% of Control
I	44.3	27.9	16.4	37.0	42.2	18.4	23.8	56.4
II	38.0	19.0	19.0	50.0	39.6	17.6	22.0	55.6
III	56.3	20.5	35.8	63.6	41.6	22.2	19.4	46.6
IV	49.4	39.4	10.0	20.2	46.1	38.0	8.1	17.6

* Figures as in Table I. † Mean of two observations.

FIG. 5.—Rate of excretion of triamterene after oral administration of 200 mg. in four normal men on a normal diet.

FIG. 6.—Effect of triamterene on sodium and potassium excretion in adrenalectomized rats. All observations refer to urine pooled from groups of three rats.

Spironolactone, even in high dosage, did not influence potassium excretion in the adrenalectomized rats, as had been already reported (Kagawa, 1960). On the other hand, triamterene produced a considerable reduction in urinary potassium output in these adrenalectomized rats.

The diuretic effect of triamterene is similar to that of chlorazaniol, a triazine derivative. Williamson *et al.* (1959) showed that chlorazaniol antagonized the salt-retaining effect of desoxycorticosterone and could inhibit its kaliuretic effect; also they found it to be effective in adrenalectomized rats.

TABLE III.—Comparison of the Effect of (A) Triamterene and (B) Spironolactone on Adrenalectomized Rats

A. Triamterene				
Group	Triamterene (mg./Rat)	Urine Vol. (ml.)	Sodium Excretion (mEq/V.)	Potassium Excretion (mEq/V.)
Control (6 groups) mean $\pm$ S.D. ..	0	17 $\pm$ 3.67	3.6 $\pm$ 0.64	0.74 $\pm$ 0.08
I	0.5	25	3.8	0.40
II	1	24	4.4	0.34
III	2	26	5.7	0.21
IV	4	28	6.1	0.20
B. Spironolactone				
Group	Spironolactone (mg./Rat)	Urine Vol. (ml.)	Sodium Excretion (mEq/V.)	Potassium Excretion (mEq/V.)
Control (6 groups) mean $\pm$ S.D. ..	0	26 $\pm$ 7.6	4.4 $\pm$ 1.24	0.74 $\pm$ 0.18
I	2	20	3.4	0.81
II	4	28	3.8	0.76
III	8	18	3.3	0.83
IV	16	36	5.8	0.72

It is clear that triamterene has a natriuretic and potassium-retaining action, demonstrable both in normal men and in adrenalectomized rats. It influences potassium excretion by a mechanism independent of aldosterone antagonism. It is probable that one of the actions of triamterene is on distal renal tubular cells, leading to a depression of exchange of sodium for hydrogen and potassium ions.

Summary

Triamterene (2,4,7-triamino-6-phenylpteridine) is effective as a diuretic agent, increasing sodium, chloride, and bicarbonate excretion and reducing potassium excretion in normal men. The degree of potassium retention was similar in normal subjects, both when on a normal diet and when receiving a high sodium intake. In adrenalectomized rats triamterene produced an increase in sodium excretion and a decrease in potassium excretion.

The results indicate that the action of triamterene is not due to aldosterone antagonism, but is probably a direct effect on the renal tubules.

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TRIAMTERENE, A NEW DIURETIC DRUG

II. CLINICAL TRIAL IN OEDEMATOUS PATIENTS

BY

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Triamterene has a diuretic effect in oedematous patients (Hild and Krueck, 1961; Laragh *et al.*, 1961; Donnelly *et al.*, 1962; Crosley *et al.*, 1962), producing an increased excretion of sodium and water but a decrease in potassium excretion. Our findings in normal subjects and in adrenalectomized rats suggested that the diuretic action of triamterene is not due to aldosterone antagonism but probably to a direct action on the renal tubular cells (Baba *et al.*, 1962).

The present work describes and compares the various types of responses encountered when hydroflumethiazide, spironolactone, and triamterene were given alone and in combination to patients with chronic salt and water retention.

Patients and Methods

Clinical studies of the action of triamterene were made in 42 patients; 27 were studied as in-patients and 15 as out-patients.

In-patient Study.—Hydroflumethiazide (100 mg.), spironolactone (400 mg.), and triamterene (200 mg.) were given alone or in combination, according to the plan illustrated in Fig. 1. All the drugs were given at 10 a.m. and urine was collected over 24-hour periods, starting at 10 a.m. each day. Before giving each dose, urine collections were made for two days, during which no drugs were given. Patients admitted to the trial were assigned alternately to Group A or Group B, the grouping determining the order of administration of the drugs. Body-weight was measured daily. All the patients took a normal diet and fluid intake was unrestricted. Blood was taken at least twice weekly for the determination of electrolytes and urea. Urine and serum creatinine levels were estimated at the beginning and end of the period of study.

Out-patient Study.—The effects of prolonged administration of triamterene alone and when combined with hydroflumethiazide or spironolactone were studied in 15 patients with chronic oedema who had all been previously treated for at least three months with oral diuretics. Observations of body weight, degree of oedema, serum electrolytes, and blood urea were made at two-week intervals, and each drug or combination of drugs was given either daily or on alternate days for periods of two weeks. Normal diet and unrestricted fluids were allowed during the trial.

Chemical Methods.—Urinary sodium and potassium were estimated by flame photometry; triamterene in urine was estimated by fluorimetry (Baba *et al.*, 1962); serum electrolytes and urea were determined by routine laboratory methods; serum and urine creatinine levels were estimated by the method of Owen *et al.* (1954).

Results

Response to Single Doses of Triamterene and of Hydroflumethiazide.—The effects of single doses of triamterene (200 mg.) and of hydroflumethiazide (100 mg.) were compared in patients in hospital. Patients of Group A received triamterene first and hydroflumethiazide second; in Group B the order was reversed. In both groups the sodium excretion was moderately increased after triamterene, but less so than after hydroflumethiazide in the doses used. Urinary potassium loss, which was increased after hydroflumethiazide was greatly reduced after triamterene (Tables I and II; Fig. 1).

Response to Combination of Triamterene and Hydroflumethiazide.—The response of hydroflumethiazide was considerably modified in both groups when triamterene was given simultaneously. There was a further increase in sodium and water excretion, whereas potassium excretion was greatly reduced. The sodium excretion and weight loss were greater than with either drug alone (Tables I, II; Fig. 1).

Comparison of Triamterene with Spironolactone.—In 20 patients the trial was continued by giving triam-

FIG. 1.—Case 4 (Group B, Table I), chronic rheumatic heart disease. Effect of hydroflumethiazide, triamterene, and spironolactone on daily urinary volume and excretion of sodium and potassium.