THE PHARMACODYNAMICS OF SINGLE DOSES OF PRORENOATE POTASSIUM AND SPIRONOLACTONE IN FLUDROCORTISONE TREATED NORMAL SUBJECTS

L.E. RAMSAY, J.R. SHELTON & M.J. TIDD

Division of Scientific Affairs, G.D. Searle & Co. Ltd,
Lane End Road, High Wycombe, Bucks HP12 4HL

1 The pharmacodynamic profile of single oral doses of prorenoate potassium (40 mg) and spironolactone (100 mg), as judged by reversal of the effects of fludrocortisone on the urinary electrolyte composition, was compared to that of placebo in a double-blind crossover study in six healthy subjects.

2 Both drugs showed evidence of significant activity in all periods between 2-16 h after treatment, and the time course of their activity was very similar.

3 In the total 24 h period after treatment both drugs significantly increased sodium excretion, the Na/K ratio and the log₁₀ Na/K ratio over placebo values, and urine potassium concentration was significantly reduced. Changes in urine volume and in potassium excretion were not significant.

4 The responses to prorenoate potassium (40 mg) were not significantly different from those to spironolactone (100 mg), and were very similar as judged by the urine log₁₀ Na/K ratio, indicating that the two drugs were approximately equiactive at these doses.

5 Reductions in urinary potassium excretion, when expressed in relation to the amount of sodium excreted, were significantly larger after prorenoate potassium than after spironolactone, confirming a qualitative difference in the pharmacological activity of the two drugs which has been reported previously.

Introduction

Prorenoate potassium is a new compound with structural similarity to spironolactone (Aldactone®, Searle), and has properties compatible with specific aldosterone antagonism in animal studies. Spironolactone has found clinical use in conditions such as congestive heart failure, hepatic ascites, primary aldosteronism and essential hypertension (Manitius & Suchecki, 1972) and prorenoate potassium is being evaluated to determine whether it may be of value in these situations. In healthy subjects single doses of prorenoate potassium have proved active in reversing the renal effects of endogenous mineralocorticoids (Levine, Ramsay, Auty, Branch & Tidd, unpublished observations) and of the synthetic mineralocorticoid fludrocortisone (Ramsay, Hessian & Tidd, 1975b). Against fludrocortisone, prorenoate potassium was significantly more active than spironolactone, with a best estimate for the relative potency (prorenoate potassium : spironolactone) of 2.7 : 1 based on the urine log₁₀ Na/K response to single doses (Ramsay, Harrison, Shelton & Tidd, 1975a). A

point of particular interest is that the potency of prorenoate potassium with respect to spironolactone as measured by retention of potassium (3.8:1) was significantly higher than its potency in promoting natriuresis (1.6:1), a finding which suggested that the pharmacological activity of the two drugs differed qualitatively (Ramsay *et al.*, 1975a).

In these previous studies against fludrocortisone the activity of prorenoate potassium and spironolactone has been assessed from changes in the electrolyte composition of urine in periods up to 10 h after treatment. Since the activity of single doses of spironolactone persists for 13-24 h (Edmonds & Wilson, 1960) we recognized that the estimates of relative potency given above might be seriously in error if there was a marked disparity in the duration of action of the two drugs. Similarly it was possible that the qualitative difference between the two drugs as regards renal handling of sodium and potassium could be artefactual, due to differences in their time-course of activity. In the present study we have treated healthy subjects

with single doses of proreanoate potassium and spironolactone which were expected to be equiactive on the basis of the previous estimate of the relative potency by the urine log 10 Na/K , (i.e. 2.7:1), and have examined in detail the time course of activity of the two drugs over a 24 h period. The method used, employing repeated doses of fludrocortisone to obtain a sustained state of mineralocorticoid agonism, is a modification and extension of that described by Edmonds & Wilson (1960).

Methods

Rationale

Fludrocortisone is a synthetic mineralocorticoid which is active, and has a prolonged duration of action, when administered orally to human subjects (Goldfien, Laidlaw, Haydar, Renold & Thorn, 1955), causing sodium retention, potassium excretion, and depression of the urinary sodium:potassium (Na/K) ratio. Its log dose-response curve exhibits parallelism with that of aldosterone (Ross, 1962) and as the latter is inactive orally and has a shorter duration of action, fludrocortisone has proved a convenient substitute for the study of aldosterone antagonists in man. In the present study the fludrocortisone dosage regime was chosen on the basis of pilot studies as one which would produce reasonably even depression of the urine Na/K ratio over a 24 h period. Activity of aldosterone antagonists is indicated by reversal of the fludrocortisone-induced urinary electrolyte changes, i.e. by increased natriuresis, decreased kaliuresis, and an increase in the urine Na/K ratio.

Subjects

Six male volunteers aged 18-35 years, judged healthy after medical history, physical examination, ECG, laboratory screening and enquiry of the family doctor, gave informed consent after full explanation of the procedure and its risks. All medications except those included in the experiment were prohibited from 1 week before the study until its completion.

Study design and procedure

Each subject was studied on three occasions with an interval of 1 week between each study period. Fludrocortisone was prescribed as detailed below on each occasion, and against this background subjects were treated with either proreanoate potassium, spironolactone or placebo, so that all

subjects received each of the three treatments once in the course of the study. The order of medication with the three treatments was balanced in a Latin square design. On the evening before study days fludrocortisone (1 mg) was given at 23.00 h, followed by 0.5 mg at 08.00 h on study days. Further doses of 0.1 mg were given at 2 hourly intervals until 22.00 h, and a final dose of 0.5 mg was given at midnight. The total dose of fludrocortisone was 2.7 mg over a 25 h period. The three trial treatments were prescribed at 08.00 h on study days, while the subjects were fasting. All urine passed in the subsequent 24 h was collected, in 2 h fractions between 0-16 h, and as a single collection between 16-24 hours. Diet and fluid intake were constant within subjects, but not between subjects, on each study day. To avoid peaks of sodium intake related to the main meals, foods of high sodium content were avoided, and salt was not used in cooking or at table. The subjects were ambulant but avoided strenuous exertion and extremes of temperature. Alcohol was prohibited during the study periods.

Drugs

Fludrocortisone (Florinef, Squibb) 1 mg and 0.1 mg tablets were prescribed open label. The trial treatments were:

- (1) proreanoate potassium 40 mg (4 x 10 mg tablets),
- (2) spironolactone 100 mg (4 x 25 mg Aldactone® tablets) and
- (3) placebo.

The treatments were dispensed double-blind using the double placebo method. These doses of proreanoate potassium and spironolactone were chosen as approximately equiactive (dose ratio 1:2.5, relative potency 2.7:1). The dose ratios in weight and molar terms are virtually identical. The potassium content of proreanoate potassium (40 mg) is 0.01 mmol.

Laboratory measurements

Urinary sodium concentration was measured by atomic absorption spectrophotometry, and potassium concentration by flame photometry using lithium as the internal standard. Laboratory screening included blood urea, electrolytes, glucose, liver function tests, haematology, urine analysis and urine microscopy, all measured by conventional laboratory procedures. Screening was performed before the study, 24 h before and after each test treatment, and 1 week after completion of the study.

Statistics

The logarithm of the urine Na/K ratio was used in analysis because this transformation stabilizes the variance and provides a more sensitive index of response in bioassays of mineralocorticoids (Johnson, 1954) and mineralocorticoid antagonists (Kagawa, 1964). The ratio was multiplied by 10 before transformation (giving $\log_{10}$ Na/K) to avoid negative values and thus simplify calculations. Responses to the three treatments were compared by analysis of variance after isolating the variance related to subjects and to phases of the study. Where the three treatments differed significantly the Newman-Keulls procedure was applied to determine which pairs of treatment means differed significantly. The changes in laboratory screen values (result 24 h after treatment minus result 24 h before treatment) were analysed similarly. Values of $P < 0.05$ in two-tailed tests were considered significant. The statistical methods used are described in Armitage (1971).

Results

The mean results in each collection period for sodium excretion, potassium excretion and urine volume are shown in Figure 1, and the mean (and s.e. mean) results for the urine $\log_{10}$ Na/K ratio are given in Table 1. It can be seen from the placebo results that the fludrocortisone regime produced fairly even depression of the $\log_{10}$ Na/K ratio throughout the 24 h period.

Pharmacodynamics of prorenoate potassium and spironolactone

The onset and duration of activity of prorenoate potassium and spironolactone were examined by comparing the responses to the three treatments within each collection period, and defining activity as a significant difference from the placebo values. In the period 0-2 h after treatment there was no evidence of activity by any parameter for either of the antagonists. In all collection intervals between 2-16 h the urine $\log_{10}$ Na/K ratio was significantly higher than placebo values after both prorenoate potassium and spironolactone (Table 1). Sodium excretion was significantly increased after both drugs in all periods between 2-14 h after treatment. The three treatments did not differ significantly as regards potassium excretion in any period. Urine volume was increased significantly at 10-12 h after spironolactone, and at 2-4 and 6-8 h after prorenoate potassium. It can be seen from the

Table 1 Mean (and s.e. mean) results of urine $\log_{10}$ Na/K for six subjects after treatment with placebo, spironolactone (100 mg) and prorenoate potassium (40 mg) at time 0. A change of 0.3 in $\log_{10}$ Na/K represents a twofold change in the urine Na/K ratio.

	Time (h)								Total 0-24
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-24
Placebo	0.63 (0.18)	0.69 (0.16)	0.67 (0.17)	0.69 (0.19)	0.64 (0.17)	0.66 (0.14)	0.72 (0.11)	0.61 (0.19)	0.50 (0.12)
Spironolactone (100 mg)	0.64 (0.10)	1.04* (0.07)	1.24** (0.07)	1.29** (0.11)	1.24** (0.05)	1.25** (0.06)	1.33** (0.01)	1.09* (0.16)	0.85 (0.05)
Prorenoate potassium (40 mg)	0.61 (0.15)	1.17* (0.15)	1.39** (0.08)	1.35** (0.12)	1.21** (0.15)	1.26** (0.09)	1.33** (0.12)	1.07* (0.16)	0.75* (0.16)

* $P < 0.05$, ** $P < 0.01$ compared to placebo values.

Figure 1 Urinary excretion of sodium and potassium and urine volume after administration of placebo (○), spironolactone (100 mg, ●) or prerenal potassium (40 mg ■) at time 0. The mean excretion rate for six subjects is plotted at the midpoint of each urine collection period.

mean values for $\log_{10} \text{Na/K}$ (Table 1) that some evidence for activity of both antagonists persisted in the 16–24 h period, although the differences did not reach significance. The minimal duration of activity for each drug may therefore be defined as extending from 2 h until 16 h after treatment. The pattern of activity of each drug within this period was examined by considering changes in the urine $\log_{10} \text{Na/K}$ ($\Delta \log_{10} \text{Na/K}$) from placebo values within each collection period.

Peak activity of prerenal potassium was observed between 4–6 h, whereas spironolactone showed a plateau of activity extending from 4–14 h, but the differences between the two drugs were very small and did not approach statistical significance at any time period. The true responses to prerenal potassium and spironolactone using the other parameters measured (Δ sodium excretion, Δ potassium excretion and Δ volume from placebo values) were also examined in each collection period. The only significant difference

between the drugs was a higher increment in urine volume for prerenal potassium at 2–4 h (for prerenal potassium mean Δ volume = +71 ml, for spironolactone mean Δ volume = +18 ml, $P < 0.05$), but we are inclined to discount this finding in view of the large number of statistical tests applied to the data.

Total responses to treatments

The responses to the three treatments over the total 24 h period were obtained by pooling the results of all the collection periods for each individual. Prerenal potassium and spironolactone each increased the $\log_{10} \text{Na/K}$ ($P < 0.01$) and sodium excretion ($P < 0.01$) above placebo values. Both drugs also increased urine volume, but not significantly ($0.05 < P < 0.1$). The three treatments did not differ significantly as regards potassium excretion ($P > 0.1$), but potassium concentration was reduced after both active treatments ($P < 0.05$). The total $\log_{10} \text{Na/K}$ responses to prerenal potassium (40 mg) (1.16 ± 0.07) and spironolactone (100 mg) (1.14 ± 0.04) were very similar, indicating that these doses were approximately equiactive by this parameter. Compared to placebo values sodium excretion was more prominent after spironolactone, whereas potassium retention was greater after prerenal potassium, but the differences between the two active drugs in these respects did not approach significance (Table 2). There was a correlation between the total response to prerenal potassium and to spironolactone in each subject as measured by $\Delta \log_{10} \text{Na/K}$ from placebo values ($r = 0.84$, $P < 0.05$), indicating that individuals with a high response to spironolactone had a similar response to prerenal potassium.

Potassium excretion following prerenal potassium and spironolactone

In view of the previous finding that prerenal potassium and spironolactone had qualitatively different effects on sodium and potassium excretion (Ramsay *et al.*, 1975a) the influence of the two drugs on potassium excretion was examined in greater depth. Using changes (Δ) from placebo values, the ratio $-\Delta K/\Delta Na$ was calculated for each drug (Table 2). This ratio represents the amount of potassium spared/mmol of sodium excreted, compared to placebo values. After prerenal potassium the urinary potassium excretion was reduced by a mean 0.40 mmol/mmol sodium, after spironolactone by 0.01 mmol/mmol sodium, and this difference was significant ($t = 2.88$, $P < 0.05$, Student's *t* for paired observations).

It can be seen from Table 2 that potassium excretion was actually higher than placebo values in three subjects after spironolactone, and in two subjects after prerenal potassium. As indicated above urinary potassium *concentration* was significantly reduced by each active drug, but the reductions in potassium *excretion* from placebo levels were small and not significant. It thus seemed that the tendency of the active drugs to conserve potassium was countered either directly or indirectly by increases in urine volume, and this was examined by correlating the changes in 24 h potassium excretion, sodium excretion and urine volume (from placebo values) for each subject. After prerenal potassium there was a significant positive correlation between Δ volume and Δ K ($r = +0.82$, $P < 0.05$, Figure 2). There was a similar but non-significant correlation between Δ Na and Δ K ($r = +0.65$, $P > 0.1$). The partial correlation of Δ volume and Δ K with Δ Na held constant remained positive ($r = +0.67$) although not significant, whereas the correlation between Δ Na and Δ K was abolished ($r = -0.07$) when Δ volume was held constant. There were no significant correlations after spironolactone, nor were the trends consistent with those after prerenal potassium.

Side-effects

The majority of subjects suffered headaches of mild-moderate severity during study days, but this symptom occurred with equal frequency after placebo and active treatments and can be attributed to the effect of fludrocortisone. There were no other side-effects, and no abnormalities of clinical importance in the laboratory tests. The

Figure 2 Correlation between changes in urine volume and potassium excretion from placebo values in the 24 h following prerenal potassium (40 mg), ($r = +0.82$, $P < 0.05$, $n = 6$).

changes in laboratory values after the three treatments were not significantly different by analysis of variance.

Discussion

Fludrocortisone has actions similar to those of aldosterone at the cellular level (Herman,

Table 2 Changes in 24 h sodium excretion (Δ Na) and potassium excretion (Δ K) from placebo values in six subjects after spironolactone (100 mg) and prerenal potassium (40 mg).

Subject	Spironolactone (100 mg)			Prerenal potassium (40 mg)		
	Δ Na (mmol)	Δ K (mmol)	$-\Delta K/\Delta Na^*$	Δ Na (mmol)	Δ K (mmol)	$-\Delta K/\Delta Na^*$
1	+ 49.2	- 41.6	+ 0.85	+ 46.8	- 42.4	+ 0.91
2	+ 73.3	+ 29.4	- 0.40	+ 109.9	+ 16.9	- 0.15
3	+ 25.1	+ 22.4	- 0.89	+ 117.0	+ 56.1	- 0.48
4	+ 154.9	+ 2.3	- 0.01	+ 52.8	- 38.6	+ 0.73
5	+ 90.5	- 45.5	+ 0.50	+ 45.4	- 25.2	+ 0.56
6	+ 147.9	- 1.3	+ 0.01	+ 88.4	- 74.6	+ 0.84
Mean	+ 90.2	- 5.7	+ 0.01	+ 76.7	- 18.0	+ 0.40**

* $-\Delta K/\Delta Na$ = amount of potassium spared/mmol sodium excreted.

** $P < 0.05$ compared to spironolactone ($t = 2.88$, paired t test).

Fimognari & Edelman, 1968) and in human subjects (Goldfien *et al.*, 1955; Thorn, Shephard, Morse, Reddy, Beigelman & Renold, 1955; Ross, 1962) and has been widely accepted as a valid substitute for aldosterone in studies using healthy subjects (Edmonds & Wilson, 1960; Ross, 1962; Noel & Leahy, 1962; Gantt & Dyniewicz, 1963; Tweeddale & Ogilvie, 1973; Ramsay *et al.*, 1975b). Its principal advantages over aldosterone are a more prolonged duration of action, and activity when administered orally, and the present study underlines its convenience as a mineralocorticoid agonist in studies of this nature. To study the activity of prorenoate potassium and spironolactone over a full 24 h period using exogenous aldosterone would have entailed the difficulty and inconvenience of prolonged intravenous infusions on three occasions to each subject. An alternative method, using stimulation of endogenous mineralocorticoids by administration of a diuretic followed by dietary sodium restriction (Edmonds & Wilson, 1960), has been used successfully to study these two drugs with results very similar to those of the present study (Levine *et al.*, unpublished observations). However, the present methodology involved less discomfort to the volunteers, and has the important advantage of requiring much less stringent dietary control so that conduct of the study on an outpatient basis, with strict supervision, was feasible. The fludrocortisone regime used appears to be satisfactory as judged by the even depression of the urine log 10 Na/K ratio over the 24 h period following placebo. Quantitatively fludrocortisone is approximately equipotent to aldosterone on a weight basis (Goldfien *et al.*, 1955; Ross, 1962), so that the subjects received doses equivalent to aldosterone (2.7 mg) over 25 hours. The mineralocorticoid state induced can be equated only crudely with that found in disease states, but was probably quite marked. Typical aldosterone secretion rates in primary aldosteronism are of the order of 0.5 mg/24 hours (Horton, 1973).

Although used as the standard drug in the present study the results for spironolactone are of some interest, since its pharmacodynamic properties after single doses have been examined in detail in only one study to our knowledge (Edmonds & Wilson, 1960), and that using the original formulation which had very low bioavailability. Our findings for spironolactone are in good agreement with those of Edmonds & Wilson (1960). There was no evidence of activity in the first 2 h after administration, and statistically significant activity persisted until 14-16 h after treatment. In clinical practice spironolactone is frequently prescribed in four

daily doses. The profile of pharmacological activity observed in this study, particularly when considered in conjunction with the multiple dose pharmacokinetics of spironolactone (Sadée, Schroder, Leitner & Dagcioglu, 1974), suggests that such frequent administration may be unnecessary, and that twelve hourly or once daily dosage with spironolactone may be equally satisfactory. The latter regimes would have the advantage of greater convenience for the patient.

The pharmacodynamic pattern observed after prorenoate potassium was very similar to that for spironolactone. There was a more distinct peak of activity between 4-6 h which was not present after spironolactone, although this difference was not statistically significant. This peak may reflect more rapid gastrointestinal absorption of prorenoate potassium, which is readily soluble in water. In contrast spironolactone has very sparing water solubility which probably limits its rate of absorption (Levy, 1962). The total 24 h response to prorenoate potassium (40 mg) measured by the urine log 10 Na/K ratio was very similar to that for spironolactone (100 mg). As the log 10 Na/K ratio is considered the best single index of antimineralocorticoid activity in studies of this nature (Kagawa, 1964; Ramsay *et al.*, 1975a), the results of the present study fully support the validity of the relative potency estimate which was derived from measurements over shorter time intervals (prorenoate potassium : spironolactone = 2.7:1) (Ramsay *et al.*, 1975a). The results also show that for each subject the response to prorenoate potassium was quantitatively similar to that for spironolactone.

We have previously suggested that there is a significant and potentially important qualitative difference in the pharmacological properties of prorenoate potassium and spironolactone, with prorenoate potassium showing relatively higher potency in conserving potassium than in promoting natriuresis (Ramsay *et al.*, 1975a). The mean results from the present study also showed a slight trend in this direction. While the drugs were convincingly equiactive by the urine log 10 Na/K ratio, spironolactone had slightly higher activity as regards sodium excretion, whereas potassium retention and the reduction in urinary potassium concentration were more marked after prorenoate potassium. These differences did not approach significance, but when the amount of potassium spared was expressed per unit of sodium excreted, prorenoate potassium was significantly more active than spironolactone. It seems clear that this finding is not an artefact due to different time courses of activity, and the results of this study therefore confirm that the two drugs differ qualitatively

under these experimental circumstances as regards their effects on renal handling of sodium and potassium. The present study is unhelpful in elucidating the mechanism of this difference, but we have suggested elsewhere that it can be explained within the framework of specific mineralocorticoid antagonism for both drugs (Ramsay *et al.*, 1975a).

The potassium sparing action of aldosterone antagonists is well established, and is a valuable property of these drugs in the clinical situation. Increases in urinary potassium excretion have been observed occasionally in the past (Romani & Keller, 1961; Jick, Morrison, Moore & Chalmers, 1963), but the pharmacological basis for this intriguing paradoxical phenomenon has not been established. An early suggestion that the increases in potassium excretion may result from increased delivery of sodium to the site of sodium: potassium exchange in the distal renal tubule (Romani & Keller, 1961) is unlikely to be correct, since more recent experimental work indicates that sodium delivery to the distal tubule is not rate-limiting for potassium secretion (Giebisch, 1971; Schultze, 1973). On the other hand it has been shown that under certain circumstances potassium secretion is a function of flow-rate through the distal tubule and possibly through the collecting ducts (Giebisch, 1971; Kunau, Webb & Borman, 1974) and this led us to examine the correlations between changes in urine volume, potassium excretion and sodium excretion in the present study. It was shown for prorenoate potassium that changes in potassium excretion could be related to changes in urine volume. This

finding suggests that the paradoxical increase in potassium excretion observed in two subjects after prorenoate potassium might be attributable to increases in urine volume, but interpretation must be guarded since the relationship did not reach significance independently of sodium excretion, and since the pattern of correlations was markedly dissimilar following spironolactone. Nevertheless we suggest that further study of the relationship between urine volume and potassium excretion in similar circumstances might prove valuable.

There were no untoward effects or laboratory abnormalities following prorenoate potassium, and the results of this study and of previous studies (Ramsay *et al.*, 1975a; Ramsay *et al.*, 1975b; Levine *et al.*, unpublished observations) suggest that evaluation of prorenoate potassium in disease states is warranted. The available evidence suggests that it may prove to be as effective as spironolactone at a dosage 1/2.7 that of the latter drug. It is recognized that increased potency is by itself trifling unless there is increased dissociation between the desired and unwanted effects (Laurence, 1964) and whether this will prove to be the case for prorenoate potassium remains to be determined.

We wish to thank Mrs M. Porteous and Mrs M. Rogers for help in conducting the study; Searle Scientific Services, who performed laboratory assays; and Miss R. Henley for secretarial help. We are grateful to the volunteers who participated in the study, and to the many personnel who have participated in the preclinical development of prorenoate potassium.

References

- ARMITAGE, P. (1971). *Statistical Methods in Medical Research*. Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications.
- EDMONDS, C.J. & WILSON, G.M. (1960). The action of hydroflumethiazide in relation to adrenal steroids and potassium loss. *Lancet*, **i**, 505-509.
- GANTT, C.L. & DYNIEWICZ, J.M. (1963). Quantitation of mineralocorticoid activity and antagonism in normal subjects. *Metabolism*, **12**, 1007-1011.
- GIEBISCH, G. (1971). Renal potassium excretion. In *The kidney, morphology, biochemistry and physiology*, eds. Ruiller, C. & Muller, A.F. **3**, pp. 329-382. New York and London: Academic Press.
- GOLDFIEN, A., LAIDLAW, J.C., HAYDAR, N.A., RENOLD, A.E. & THORN, G.W. (1955). Fluorohydrocortisone and chlorohydrocortisone, highly potent derivatives of compound F. *New Engl. J. Med.*, **252**, 415-421.
- HERMAN, T.S., FIMOIGNARI, G.M. & EDELMAN, I.S. (1968). Studies on renal aldosterone-binding proteins. *J. biol. Chem.*, **243**, 3849-3856.
- HORTON, R. (1973). Aldosterone: review of its physiology and diagnostic aspects of primary aldosteronism. *Metabolism*, **22**, 1525-1545.
- JICK, H., MORRISON, R.S., MOORE, E.W. & CHALMERS, T.C. (1963). Metabolic studies on the effects of spironolactone. *Metabolism*, **12**, 381-387.
- JOHNSON, B.B. (1954). Bioassay of adrenal cortical steroids on the basis of electrolyte excretion by rats. Effects of 11-desoxy and 11-oxy steroids. *Endocrinology*, **54**, 196-208.
- KAGAWA, C.M. (1964). Adrenocortical antagonists. In *Evaluation of drug activities: pharmacometrics*, eds. Laurence, D.R. & Bacharach, A.L., **2**, pp. 745-762. London and New York: Academic Press.
- KUNAU, R.T., WEBB, H.L. & BORMAN, S.C. (1974). Characteristics of the relationship between the flow rate of tubular fluid and potassium transport in the distal tubule of the rat. *J. clin. Invest.*, **54**, 1488-1495.
- LAURENCE, D.R. (1964). First clinical trials of potential drugs. In *Evaluation of drug activities: pharmacology*.

- metrics, eds. Laurence, D.R. & Bacharach, A.L., 2, pp. 3-22. London and New York: Academic Press.
- LEVY, G. (1962). Availability of spironolactone given by mouth. *Lancet*, ii, 723-724.
- MANITIUS, A. & SUCHECKI, T. (1972). The mode of action and clinical use of spiro lactones. *Materia med. Polona*, 4, 83-88.
- NOEL, P.R. & LEAHY, J.S. (1962). The estimation of the activity of aldosterone antagonist in man: Spironolactone (Aldactone) activity. *Clin. Sci.*, 23, 477-483.
- RAMSAY, L., HARRISON, I., SHELTON, J. & TIDD, M. (1975a). The relative potency of prorenoate potassium and spironolactone in normal man. *Clin. Pharmac. Ther.*, 18, 391-400.
- RAMSAY, L.E., HESSIAN, P. & TIDD, M.J. (1975b). Bioassay of aldosterone antagonists in normal human subjects; a relationship between the level of plasma uric acid before treatment and apparent drug responses. *Br. J. clin. Pharmac.*, 2, 271-276.
- ROMANI, J.D. & KELLER, A. (1961). Etude des effets metaboliques de la spiro lactone SC 9420 chez l'homme. *Annls. Endocr.*, 22, 357-366.
- ROSS, E.J. (1962). Human assay of electrolyte-active steroids and their antagonists. *Clin. Sci.*, 23, 197-202.
- SADEE, W., SCHRODER, R., LEITNER, E. & DAGCIOGLU, M. (1974). Multiple dose kinetics of spironolactone and canrenoate - potassium in cardiac and hepatic failure. *Eur. J. clin. Pharmac.*, 7, 194-200.
- SCHULTZE, R.G. (1973). Recent advances in the physiology and pathophysiology of potassium excretion. *Archs. intern. Med.*, 131, 885-897.
- THORN, G.W., SHEPPARD, R.H., MORSE, W.I., REDDY, W.J., BEIGELMAN, P.M. & RENOLD, A.E. (1955). Comparative action of aldosterone and 9-alpha-fluorohydrocortisone in man. *Ann. N.Y. Acad. Sci.*, 61, 609-619.
- TWEEDDALE, M.G. & OGILVIE, R.I. (1973). Antagonism of spironolactone-induced natriuresis by aspirin in man. *New Engl. J. Med.*, 289, 198-200.

(Received June 28, 1975)