PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184,** 1–6 (1987)

Potassium-Sparing Effect of Trilostane in Hydrochlorothiazide-Treated Rats and Dogs (42437)

ALBERT F. DEFELICE,[1] ARMAND C. BROUSSEAU, AND BERNARD O'CONNOR

Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144

Abstract. Trilostane, which inhibits three β-hydroxysteroid dehydrogenase and aldosterone synthesis in rats and monkeys, significantly attenuated the oral potassium-wasting effect of hydrochlorothiazide (HCTZ) in rats and dogs when coadministered with the diuretic. The steroid reduced the kaliuretic and enhanced the natriuretic (and hyperreninemic) activity of HCTZ in rats, thereby promoting the urinary sodium/potassium ratio. Trilostane completely prevented HCTZ-induced hypokalemia in dogs and tended to reduce the degree of secondary aldosteronism. The combination also promoted hematocrit of dogs by 8% and decreased serum Na^+ concentration by 7 meq/liter. When administered alone, trilostane increased canine serum potassium levels slightly and promoted rat urinary Na^+/K^+ ratio. Results confirm previous reports of antikaliuretic activity of trilostane in diuretic-treated rats. Further, the data indicate that frank hypokalemia induced in dogs by hydrochlorothiazide can be prevented by adjunctive trilostane therapy without eliciting hyperkalemia. © 1987 Society for Experimental Biology and Medicine.

Thiazides and other potent natriuretic agents promote renal potassium excretion which induces varying degrees of hypokalemia in patients (1, 2). The increased potassium loss is attributed primarily to augmented delivery of sodium and fluid to distal tubule sites where potassium secretion is sodium dependent and to enhanced secretion of aldosterone which facilitates distal sodium absorption and potassium secretion (3). Accordingly, inhibiting the activity of aldosterone is a rational approach to reversing or attenuating such reductions in serum K^+ levels. Indeed, spironolactone, an aldosterone receptor antagonist, inhibits the potassium wasting and also enhances the antihypertensive activity of the thiazide diuretics (4, 5). Inhibitors of aldosterone synthesis could have similar utility. (4α, 5α, 17β)-4,5-Epoxy-3,17-dihydroandrast-2-ene-2carbonitrile (trilostane), which is approved for treating Cushing's syndrome, inhibits 3 β-hydroxysteroid dehydrogenase, suppresses aldosterone production *in vivo* in sodium-deprived rats, and lacks hormone agonist or antagonist activity (6). Trilostane also prevents furosemide-induced hyperaldosteronism and kaliuresis in rats without suppressing its natriuretic and diuretic activity (7).

This study assesses oral inhibitory effects of trilostane on hydrochlorothiazide-induced kaliuresis and hypokalemia in rats and dogs and whether such effects could relate to depressed plasma renin activity and serum aldosterone levels.

Materials and Methods. *Renal electrolyte escretion and plasma renin levels in rats.* Male Sprague–Dawley rats (160–200 g, Taconic Farms), housed with a 12-hr light–dark cycle and fed Wayne Lab-Blox (Continental Grain Co., Chicago, Ill.) were fasted overnight, bladders were emptied by gentle suprapubic pressure, and the animals were weighed. Rats were then placed in individual metabolism cages equipped for urine collection. Trilostane (Sterling–Winthrop Research Institute, Rensselaer, N.Y.) and hydrochlorothiazide (HCTZ; CIBA Pharmaceutical, Summit, N.J.) were suspended in 1% aqueous gum tragacanth and administered alone or combined in a volume of 30 ml/kg body wt at $t = 0$ and again at $t = 2\frac{1}{2}$ hr. Rats received 100 mg/kg of trilostane and/or 30 mg/kg of HCTZ each time. Control rats received vehicle alone. Urine was collected for 5 hr, and the 0- to $2\frac{1}{2}$-hr and $2\frac{1}{2}$- to 5-hr samples were segregated. Each sample contained spontaneously voided urine and that obtained by expressing the bladder and washing the cages at $t = 2\frac{1}{2}$ or 5 hr. Rats were decapitated at $t = 5$ hr, 2 ml of blood was obtained, and plasma was assayed for renin

[1] To whom correspondence should be addressed.

0037-9727/87 $1.50
Copyright © 1987 by the Society for Experimental Biology and Medicine.
All rights reserved.

concentration (PRC) expressed as rate of angiotensin I generation.

Serum electrolyte, hematocrit, and steroid levels in dogs. Beginning 14 days prior to dosing, male and female beagle hounds (9–12 kg, Marshall's Animal Research, North Rose, N.Y.) were fed daily with 28 g/kg of Wayne Lab dog diet which contained 0.22 meq of sodium and 0.13 meq of potassium per gram. Tap water was allowed *ad libitum.* Venous blood for baseline determination was drawn at 8:00 AM on Days 10, 13, and 15. Hematocrit was determined by centrifugation, and the serum was stored at −15°C. Trilostane and HCTZ, alone or combined, were then administered twice daily (8:30 AM and 4:00 PM) in gelatin capsules for 9 consecutive days. Treated dogs received 25 mg/kg of trilostane and/or 4.5 mg/kg of HCTZ each time. Venous blood drawn at 11:00 AM from these and lactose-treated control dogs on Days 17, 20, and 23 (i.e., 3, 6, and 9 days post-treatment) was also centrifuged, hematocrit was measured, and the serum was frozen. Serum samples obtained during equilibration and treatment periods were thawed and assayed for sodium, potassium, cortisol, and aldosterone concentrations.

Biochemical determinations. Sodium and potassium levels in rat urine and canine plasma were measured with a flame photometer (Instrumentation Labs, Watertown, Mass., Model 243). Urine values were calculated as microequivalents excreted in the 0- to $2\frac{1}{2}$-hr, $2\frac{1}{2}$- to 5-hr, and 0- to 5-hr collection periods. Rat plasma, thawed on ice, was mixed with angiotensinogen-rich plasma from nephrectomized rats (8) and assayed at 37°C for plasma renin concentration (expressed as ng angiotensin I/ml plasma/hr) using an angiotensin I radioimmunoassay (New England Nuclear Corp., No. Billerica, Mass.). Dog plasma aldosterone and cortisol levels were determined by radioimmunoassay (Diagnostic Products Corp., Los Angeles, Calif.; New England Nuclear Corp.).

Statistical analysis. Analysis of variance, followed by Duncan's multiple-range or Tukey's studentized range test and Student's *t* test were used to identify treatment effects. For the dog study, mean of individual changes from baseline during treatment period (i.e., mean (Days 17, 20, 23) - mean (Days 10, 13, 15)) was compared to corresponding change in placebo group. Linear regression analysis was performed using a computer program published by Tallarida (9).

Results. *Rat studies.* Profiles of urinary electrolyte excretion of the hydrated rats in the 5-hr observation period are shown in Tables I and II. Vehicle-treated rats excreted an average of 0.45 and 1.34 meq of Na^+ and K^+, respectively. Hydrochlorothiazide promoted absolute N^+ and K^+ excretion to approximately 12× and 3× control values (Table I). This was due primarily to increased electrolyte concentration as urine volume of the water-loaded rats was not significantly enhanced. Trilostane, used adjunctively, reduced potassium excretion of HCTZ-treated rats by 25% (2.73 *vs* 3.62 meq) without depressing natri-

TABLE I. ORAL ANTI-KALIURETIC ACTIVITY OF TRILOSTANE IN HYDROCHLOROTHIAZIDE-TREATED RATS

	Urine (5-h collection)[b]						
		Sodium		Potassium			
Treatment[a]	Volume (ml)	(meq)	(meq/dl)	(meq)	(meq/dl)	Na^+/K^+	Plasma renin concentration (ng AI/ml/hr)[b]
Vehicle	8.1 ± 0.5	0.45 ± 0.08	0.05 ± 0.01	1.34 ± 0.14	0.17 ± 0.02	0.35 ± 0.08	16.1 ± 2.3
HCTZ	10.7 ± 0.6	5.21 ± 0.43[c]	0.5 ± 0.05[c]	3.62 ± 0.32[c]	0.35 ± 0.04[c]	1.45 ± 0.08[c]	27.3 ± 2.6[c]
Trilostane	8.6 ± 0.4	1.56 ± 0.14[c]	0.18 ± 0.01[c]	0.99 ± 0.09	0.12 ± 0.02[c]	1.62 ± 0.18[c]	22.0 ± 1.6
HCTZ and trilostane	10.1 ± 0.6	5.97 ± 0.21[c]	0.61 ± 0.08[c]	2.73 ± 0.41[c,d]	0.28 ± 0.06	2.37 ± 0.26[c,d]	36.5 ± 4.5[c,d]

[a] Trilostane and hydrochlorothiazide (HCTZ) were administered orally at doses of 100 and 30 mg/kg, respectively, at 0 and $2\frac{1}{2}$ hr.

[b] Mean ± SE of six rats/group.

[c] Significantly different from the mean of the group treated with vehicle alone ($P < 0.05$).

[d] Significantly different from the mean of the group treated with HCTZ alone ($P < 0.05$).

TABLE II. TIME COURSE OF ORAL ANTI-KALIURETIC ACTIVITY OF TRILOSTANE IN HYDROCHLOROTHIAZIDE-TREATED RATS

Treatment[a]	Urine[b]								
	0- to 2 ½-hr collection				2½- to 5-hr collection				
	Volume (ml)	Sodium		Potassium		Volume (ml)	Sodium		Potassium	
		(meq)	(meq/dl)	(meq)	(meq/dl)		(meq)	(meq/dl)	(meq)	(meq/dl)
Vehicle	3.8 ± 0.6	0.29 ± 0.05	0.09 ± 0.01	0.78 ± 0.13	0.28 ± 0.10	4.3 ± 0.2	0.16 ± 0.05	0.04 ± 0.01	0.56 ± 0.07	0.14 ± 0.02
HCTZ	6.2 ± 0.5	4.0 ± 0.39[c]	0.68 ± 0.12[c]	2.53 ± 0.34[c]	0.43 ± 0.09	4.5 ± 0.3	1.20 ± 0.15[c]	0.27 ± 0.02[c]	1.09 ± 0.08[c]	0.25 ± 0.02[c]
Trilostane	3.9 ± 0.4	0.64 ± 0.09[c]	0.18 ± 0.04	0.71 ± 0.07	0.20 ± 0.04	4.7 ± 0.2	0.92 ± 0.15[c]	0.20 ± 0.03[c]	0.28 ± 0.05[c]	0.06 ± 0.01[c]
HCTZ and trilostane	5.9 ± 0.6	3.74 ± 0.36[c]	0.73 ± 0.19[c]	2.27 ± 0.38[c]	0.44 ± 0.13	4.3 ± 0.3	2.24 ± 0.22[c,d]	0.53 ± 0.04[c,d]	0.47 ± 0.05[d]	0.11 ± 0.01[d]

[a] Trilostane and HCTZ were given at doses of 100 and 30 mg/kg, respectively, at 0 and 2 ½ hr.
[b] Mean ± SE of six rats/group
[c] Significantly different from the mean of the group treated with vehicle alone ($P < 0.05$)
[d] Significantly different from the mean of the group treated with HCTZ alone ($P < 0.05$)

uretic activity of the thiazide. The steroid promoted basal (Table I) as well as HCTZ-stimulated sodium excretion (Table II: 2½ to 5-hr interval) by 1.11 and 1.04 meq (246 and 87%), respectively. Accordingly, urine Na^+/K^+ ratios of both groups of rats treated with the steroid were elevated. Potassium-sparing activity of trilostane was manifested in the 2½- to 5-hr period, where it potentiated the natriuretic and abolished the kaliuretic effects of HCTZ, and reduced basal K^+ excretion by 50%. The modest natriuretic effect of trilostane per se was evident throughout the 5-hr observation.

Rates of urinary sodium excretion and plasma renin levels were positively correlated. Quantity of sodium excreted by trilostane, HCTZ, and trilostane + HCTZ-treated rats was 3.5×, 11.6×, and 13.3× control levels ($P < 0.05$ for all effects), while respective plasma renin concentrations were 1.4× ($P > 0.05$), 1.7× ($P < 0.05$), and 2.3× ($P < 0.05$) placebo values (Table I). Regression analysis of sodium excretion vs plasma renin activity for all four groups yielded a correlation coefficient (r) of 0.637 ($P < 0.01$). Slope of the line generated by least-squares analysis was significantly different ($P < 0.05$) from 0.

Dog studies. The four dog groups had similar mean pretreatment levels for K^+ and Na^+ (4.38 to 4.48 and 144 to 145 meq/liter, respectively), aldosterone and cortisol (73 to 95 pg/ml and 23 to 30 ng/ml, respectively), and hematocrit (47 to 49% packed RBC) (Table III). Plasma potassium and sodium concentrations are shown in Figs. 1 and 2. Plasma K^+ levels stabilized within 14 days of dietary adaptation and did not change during subsequent treatment with lactose for 9 days. Mean K^+ levels were reduced by 19% (0.70 μeq/ml) during 9 days treatment with HCTZ (4.5 mg/kg, twice daily). Coadministration of trilostane completely prevented HCTZ-induced hypokalemia. Tolerance to the prophylactic effect did not occur. Trilostane administered alone raised mean plasma K^+ levels by 0.27 μeq/ml, i.e., 6%. The combination of trilostane and HCTZ depressed plasma Na^+ levels by 6.5 meq/liter (5%) and increased hematocrit by 8% (Figs. 2, 3). Trilostane per se depressed plasma Na^+ slightly (approximately 2.5 meq/liter; 2%).

Plasma aldosterone changes are given in Fig. 4. HCTZ increased mean aldosterone levels

TABLE III. BASELINE CANINE HEMATOCRIT AND PLASMA ELECTROLYTE AND STEROID LEVELS[a]

Treatment group[b]	K^+ (meq/liter)	Na^+ (meq/liter)	Hematocrit (% packed RBC)	Aldosterone (pg/ml)	Cortisol (ng/ml)
Lactose	4.38 ± 0.03	144 ± 1.0	49 ± 1	75 ± 15	27 ± 2
HCTZ	4.46 ± 0.06	144 ± 0.2	47 ± 1	94 ± 5	30 ± 4
Trilostane	4.43 ± 0.04	144 ± 0.5	47 ± 1	95 ± 20	23 ± 2
HCTZ and trilostane	4.48 ± 0.10	145 ± 0.4	48 ± 1	73 ± 5	23 ± 2

Note. N = 6 dogs/group.
[a] Mean (±SE) for Days 10, 13, and 15.
[b] Trilostane and HCTZ were subsequently given orally at doses of 25 and 4.5 mg/kg, respectively, twice daily.

two- to fourfold during 9 days of treatment. Inclusion of trilostane tended to reduce the extent of the aldosteronism ($P = 0.13$), although mean plasma aldosterone levels of combination-treated dogs still exceeded those of controls.

No significant changes in plasma cortisol levels were observed during the treatment period (differences between treatment and baseline values for the lactose, HCTZ, trilostane, and combination groups were +4, −2, −5, and −3 ng/ml, respectively).

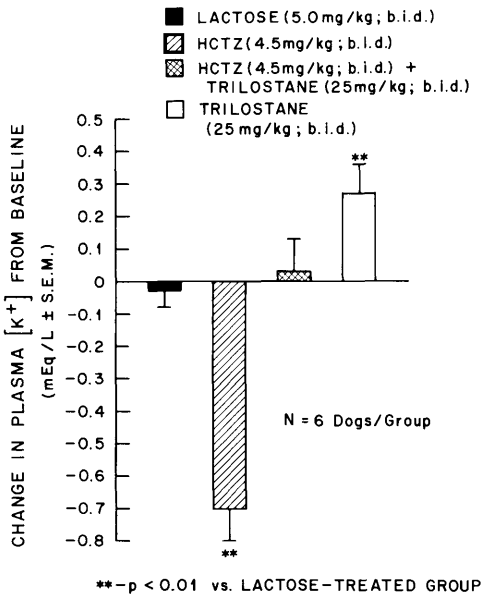

FIG. 1. Oral effect of trilostane on hydrochlorothiazide (HCTZ)-induced hypokalemia. Dogs were fed a controlled diet for 23 days and treated on Days 15 through 23 at 8:00 AM and 4:00 PM. Differences in plasma K^+ levels from pretreatment baseline, i.e., mean (Days 17, 20, 23) − mean (Days 10, 13, 15) are shown. Blood was drawn at 8:00 AM during control period and at 11:00 AM during test period.

Discussion. Results indicate that hydrochlorothiazide enhances urinary potassium loss and produces frank hypokalemia, hyperreninemia, and aldosteronism in rats or dogs, as in man, to complicate natriuretic therapy. Coadministration of trilostane, a potent inhibitor of 3-β-hydroxysteroid dehydrogenase, attenuated thiazide kaliuresis in rats and completely prevented hypokalemia in dogs with no indication of depressed natriuresis or diuresis. Therefore, trilostane may be a useful adjunct to thiazide therapy to prevent muscle weakness, ventricular ectopic activity, and other sequelae of potassium wasting while preserving or enhancing diuresis and natriuresis.

Several mechanisms have been invoked for hydrochlorothiazide-induced kaliuresis and hypokalemia. Rat urinary potassium appears

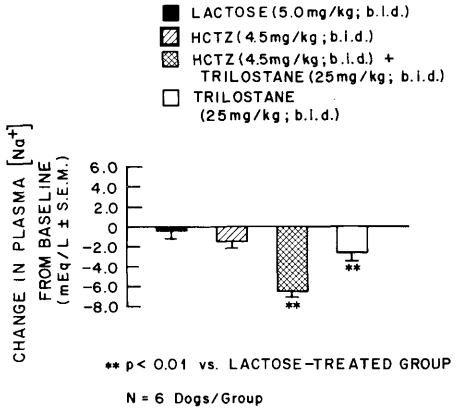

FIG. 2. Oral effect of trilostane and hydrochlorothiazide on plasma sodium concentration in conscious dogs. See legend to Fig. 1 for protocol.

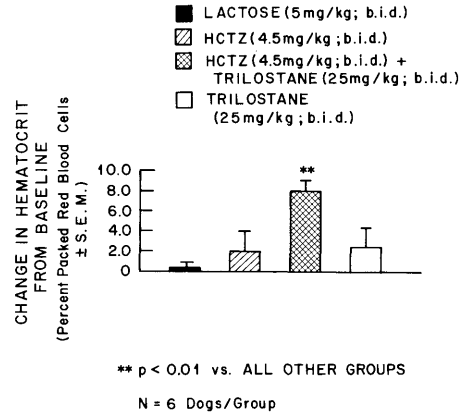

FIG. 3. Influence of trilostane and hydrochlorothiazide on whole blood hematocrit of conscious dogs. See legend to Fig. 1 for protocol.

to be derived primarily from ion secreted into the distal convoluted tubule lumen in exchange for reabsorbed sodium (10, 11). Accordingly, this secretion is influenced by the load of sodium reaching the distal tubule, and is enhanced when more sodium is presented to the Na^+–K^+ exchange sites. Increased delivery of sodium to the distal tubule to facilitate potassium secretion is a major factor in thiazide kaliuresis, and resultant hypokalemia, in man, rats, and dogs (10, 11). As shown in beagles, HCTZ also stimulates release of aldosterone which facilitates tubular Na^+–K^+ exchange. Hydrochlorothiazide also inhibits carbonic anhydrase (10), which is expected to inhibit hydrogen ion secretion and to promote potassium secretion. However, this is believed to influence potassium secretion less than the increased delivery of sodium to the distal tubule (12).

Accordingly, the potassium-sparing effect of trilostane, now confirmed in rats and extended to the beagle, could be achieved via several mechanisms. Antagonism of the natriuretic effect of the thiazide in the rat can be excluded since urinary sodium excretion was actually enhanced by combination therapy. In beagles as well, the slight hyponatremia and elevated hematocrit observed when trilostane and HCTZ are coadministered is consistent with enhanced sodium excretion and hemoconcentration. Alternatively, the steroid may directly inhibit distal tubular sodium–potassium exchange or, like spironolactone, block the facilitatory effect of aldosterone on this process. Either of these actions would be consistent with the reciprocal effects of trilostane on sodium and potassium excretion reported herein. Evidence against either direct or spironolactone-like effects on ion exchange exists in rats. After adrenalectomy, trilostane no longer inhibits furosemide kaliuresis (7) nor, unlike spironolactone, can it prevent deoxycorticosterone-induced potassium loss. Rather, it is likely that trilostane blunts hydrochlorothiazide-induced aldosteronism although a statistically significant reduction was not observed in the present studies. However, plasma potassium and aldosterone levels were inversely correlated only in dogs treated with HCTZ alone. Further, trilostane per se raised plasma potassium without depressing basal aldosterone levels, which indicates that these parameters can vary independently. More studies are required to clarify any dependency of plasma potassium concentration on circulating (or distal tubular) aldosterone levels during chronic diuretic therapy, and to confirm a statistically significant effect of trilostane on such levels, before invoking an antisteroidogenic mechanism for the potassium-conserving effect of this drug. The antikaliuretic effect of trilostane could be achieved by

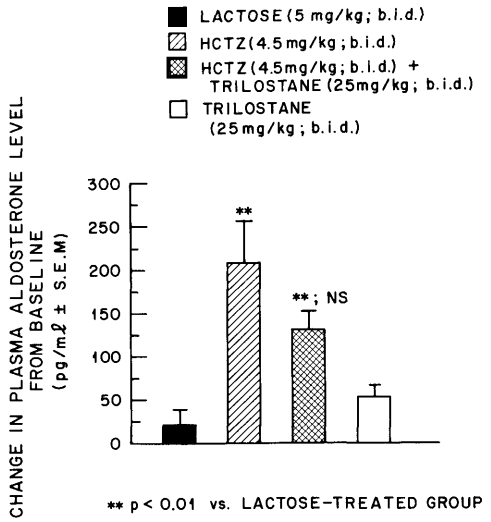

FIG. 4. Influence of hydrochlorothiazide and trilostane on plasma aldosterone concentration in conscious dogs. See legend to Fig. 1 for protocol. N. S., not significantly different ($P = 0.13$) from group treated with HCTZ alone.

depressing renin secretion and, thereby, plasma angiotensin levels which could interfere with angiotensin-stimulated aldosterone secretion (13). The potassium-sparing effect, at least in the rat, is not secondary to depression of plasma renin activity since the latter was stimulated by the thiazide in the presence or absence of trilostane.

Hypokalemia is the most common side effect of diuretic therapy (14). It can precipitate potentially harmful side effects including ventricular ectopy, ileus, digoxin toxicity, and renal tubular damage (15). Hypokalemia can be difficult to correct owing to serious limitations of current treatments. For example, potassium salt supplements are unpalatable, irritating, and often do not restore potassium balance (16). Spironolactone effectively conserves potassium but produces gastrointestinal and endocrine dysfunction in up to 20% of patients (17). The results of the present study indicate that trilostane could provide another means of preventing and possibly reversing diuretic-induced hypokalemia.

The authors thank Ms. Elaine Garee, Mr. Edward Cushine, and Ms. Kathleen Arthur for performing biochemical and statistical analyses.

1. McMahon F. Efficacy of antihypertensive agent comparison of alpha-methyldopa plus hydrochlorothiazide in combination and singly. J Am Med Assoc **231**;155–165, 1975.
2. Pederson O. Comparison of metoprolol and hydrochlorothiazides as antihyper tensive agents. Eur J Clin Pharmacol **10**(6):381–394, 1976.
3. Suki W, Stinebaugh B, Frammer J, Eknoyan G. Physiology of diuretic action. In: Seldin D, Giebish G, eds. The Kidney: Physiology and Pathophysiology. New York, Raven Press, Vol 2:p2145, 1985.
4. Fagerberg S, Goransson K. A comparative study of spironolactone and chlorthalidone in treatment of hypertension. Lakartidningen **71**:175–177, 1974.
5. Berglund G, Anderson, O. Hydrochlorothiazide and spironolactone alone and in fixed combination in hypertension. Curr Ther Res **27**(3):360–364, 1980.
6. Potts G, Creange J, Harding H, Schane H. Trilostane, an orally active inhibitor of steroid biosynthesis. Steroids **32**:257–267, 1978.
7. Harding H, Creange J, Potts G, Schane HP. Inhibition of furosemide-induced kaliuresis in the rat by trilostane, an active inhibitor of adrenal steroidogenesis. Proc Soc Exp Biol Med **177**:388–391, 1984.
8. Menard J, Catt K. Measurement of renin activity, concentration, and substrate in rat plasma by radioimmunoassay of angiotensin I. Endocrinology (Baltimore) **90**:422–430, 1972.
9. Tallarida R, Murray R. Manual of Pharmacologic Calculations with Computer Programs. New York, Springer-Verlag, pp 9–12, 1981.
10. Mudge G. Drugs affecting renal function and electrolyte metabolism. In: Goodman L, Gilman A, eds. The Pharmacological Basis of Therapeutics. New York, Macmillan Co., 4th ed. pp855–862, 1970.
11. Bank N. Physiological basis of diuretic action. Annu Rev Med **19**:103–118, 1968.
12. Berliner R. Use of modern diuretics. Circulation **33**: 802–809, 1966.
13. Davis J. A critical evaluation of the role of receptors in the control of aldosterone secretion and sodium excretion. Prog Cardiovasc Dis **4**:27–46, 1961.
14. Gifford R. A guide to the practical use of diuretics. J Amer Med Assoc **235**:1890–1893, 1976.
15. Griffing G, Melby J. The therapeutic use of a new potassium-sparing diuretic, amiloride, and a converting enzyme inhibitor, MK-421, in preventing hypokalemia associated with primary and secondary hyperaldosteronism. Clin Exp Hyper **A5**(6):779–801, 1983.
16. Whang R, Aikawa J. Magnesium deficiency and refractoriness to potassium repletion. J Chron Dis **30**: 65–68, 1977.
17. Greenblatt D, Koch-Weser J. Adverse reactions to spironolactone. A report from the Boston collaborative drug surveillance program. J Amer Med Assoc **255**: 40–43, 1973.

Received May 30, 1986. P.S.E.B.M. 1987, Vol. 184.
Accepted September 9, 1986.