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Effect of Furosemide on Experimental Hypercalcemia in Dogs (37783)

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(Introduced by D. Massaro)

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The effect of diuretics on renal transport of calcium and magnesium has recently received considerable attention (1–4). Of the agents examined the most pronounced increases in calcium and magnesium excretion occur with furosemide and ethacrynic acid Because of the limitations of existing symptomatic therapy for clinical hypercalcemia, Suki *et al.* (5), tried furosemide for the acute treatment of this disorder in eight patients with satisfactory results. In a more recent study, furosemide diminished the hypercalcemic response of rats to dihydrotachysterol when both agents were given concurrently (6). This investigation was undertaken to examine the actue and chronic effects of furosemide on experimental hypercalcemia in dogs in an attempt to define conditions for its application in this disorder.

Methods. Acute and chronic studies were performed on female mongrel dogs with experimental hypercalcemia. The experimental models were as follows:

a. Acute furosemide administration. Fifteen experiments were performed on six dogs weighing between 16 and 30 kg. The animals were maintained on commercial dog food and were allowed free access to water up to the morning of the experiment. Hypercalcemia was induced by the oral administration of 150,000 units of calciferol 5 to 6 times weekly and 4–5 g of calcium chloride added to the diet. When serum calcium levels reached 13–15 mg%, studies were performed on each animal in accordance with the following experimental design. After an overnight fast, the dogs were anesthetized intravenously with sodium pentobarbital (25 mg/kg body wt) and catheterized with an indwelling bladder catheter. They were ventilated on room air through an endotracheal tube connected to a Harvard respirator. Priming and sustaining doses of inulin in normal saline were infused to achieve a calculated plasma concentration of 20–30 mg%. The sustaining infusion was given at a rate of 1.7 ml/min. After a 50–60 min period of equilibration, 3 control periods of 20 min duration were collected. The animal was then given an intravenous injection of furosemide in a dose of 5 mg/kg body weight followed by a sustaining infusion of 5 mg/kg body weight/hr for the rest of the experiment. Isotonic saline was then begun and the rate of infusion was adjusted to equal urine flow rate as closely as possible to prevent depletion of extracellular fluid volume (ECF). One hour after the start of furosemide 3 experimental periods of 20 min duration were collected. Blood was collected at the midpoint of each period from an indwelling needle in the femoral artery and water/air bladder washouts were performed to insure complete urine collections when flow was less than 2 ml/min. Two or three experiments were performed on each animal at intervals of not less than 1 wk on the same dog.

b. Chronic furosemide administration. Ten dogs weighing between 8 and 21 kg were started on the study. They were housed in metabolic cages and offered a daily diet consisting of 16 to 32 oz of commercial horsemeat with free access to water and dog biscuits. In an attempt to achieve a condition of

chronic, stable hypercalcemia of 13–15 mg%, we found it necessary to resort to the subcutaneous administration of parathyroid hormone (PTH) at 12 hr intervals. This dose which varied between 100 and 400 units daily was then continued throughout the remainder of the experiment. When fairly stable hypercalcemia was achieved for at least 3 days, furosemide in doses of 2.5 to 15 mg/kg body weight was given intramuscularly twice daily for 4 to 7 days. During furosemide administration, supplemental sodium chloride in amounts approximating the previous day's urinary excretion was mixed with the animal's meat ration. This ranged from 6 to 20 g daily. In those animals which became anorexic even to the point of refusing hand feeding, saline was given intravenously to replace furosemide-induced losses. A post-furosemide recovery period of 2 to 7 days completed the study. Weights, venous bloods, and 24 hr urines were obtained daily throughout each phase of the investigation. Serum nitrogen (SUN) was measured at 5- to 7-day intervals.

Sodium and potassium were measured by flame photometry and calcium and magnesium by atomic absorption spectrophotometry. Analyses on inulin and urea were performed by autoanalyzer. Inulin clearance and electrolyte excretion rates were calculated by standard formulae as was statistical analysis of the data (7).

Results. Acute experiments. A representative acute experiment is shown in Table I. Control levels of plasma calcum (P_{Ca}) ranged between 13.8 and 14.3 mg% with a base-line calcium excretion rate of 60 μg/min. Following the administration of furosemide, there was a striking diuretic response with large increases in the excretion of sodium, potassium, calcium and magnesium. This was associated with a significant increase in inulin clearance and a decrease of P_{Ca} and magnesium (P_{Mg}). Data from each acute experiment are summarized in Table II. Each value represents the average of 3 control (C) or 3 experimental periods (E). In each experiment, furosemide induced an obvious diuretic response which varied in intensity from dog to dog and in the same dog from experiment

TABLE I. Representative Experiment Showing Acute Effects of Furosemide and Concurrent Volume Repletion on Hypercalcemic Dog B.

Time (min)	V (ml/min)	C_{In} (ml/min)	P_{Na} (mEq/liter)	$U_{Na}V$ (μEq/min)	P_K (mEq/liter)	U_KV (μEq/min)	P_{Ca} (mg%)	$U_{Ca}V$ (μg/min)	P_{Mg} (mg%)	$U_{Mg}V$ (μg/min)
−15	Sodium pentobarbital, 25 mg/kg iv (18 kg mongrel dog)									
0	Prime: Inulin, 840 mg; start Infusion I: Inulin, 9 mg/min in 0.9% NaCl solution at 1.7 ml/min									
60–80	0.3	19.1	138	6.0	2.9	9.0	14.3	60	1.5	30
80–100	0.3	15.3	138	5.0	2.9	8.0	14.2	60	1.5	30
100–120	0.3	13.7	138	6.0	2.9	9.0	13.8	60	1.5	30
120	Prime: Furosemide, 5 mg/kg; add furosemide, 5 mg/kg/hr to Infusion I									
125	Infusion II: 0.9% NaCl intravenously to approximate urinary output for remainder of experiment									
180–200	19.4	33.0	142	2625	2.6	78	10.5	1400	1.0	210
200–220	20	33.4	142	2710	2.6	80	9.8	970	1.0	140
220–240	20.5	33.4	142	2798	2.6	82	9.5	1320	0.9	180

TABLE II. Summary of Experiments Showing the Acute Effects of Furosemide on Hypercalcemic Dogs.

DOG	V (ml/min)		C_{IN} (ml/min)		$U_{Na}V$ (μEq/min)		U_KV (μEq/min)		P_{Ca} (mg%)		$U_{Ca}V$ (μg/min)		P_{Mg} (mg%)		$U_{Mg}V$ (μg/min)	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
	0.53	14.1	16.8	30	7.4	1944	18.0	68	16.1	13.5	80	1110	1.96	1.47	60	250
A	0.35	4.0	7.6	9.4	6.5	520	13.7	32	12.3	11.2	10	230	1.97	1.72	30	100
	0.29	6.9	21.2	21.1	11.6	930	15.3	48	12.5	12.2	100	610	1.47	1.11	70	120
	0.31	7.7	20.7	18.6	9.7	1018	22.4	56	14.7	12.1	80	760	1.54	1.14	40	100
B	0.48	21.2	18.8	37.2	5.3	2791	9.0	117	15.3	9.6	130	1430	1.80	1.07	70	230
	0.30	20.0	16.0	33.3	5.6	2711	8.7	80	14.1	9.9	60	1230	1.50	0.96	30	180
	0.53	39.8	63.2	89.6	20.9	5315	13.0	203	14.4	11.8	290	2730	1.28	0.85	110	410
C	0.38	32.8	52.0	73.9	13.6	4691	12.0	181	13.9	8.5	90	3100	1.29	0.67	90	300
	0.27	18.0	48.1	66.5	7.3	2460	9.0	111	12.6	11.4	90	1120	1.31	0.96	70	240
D	0.89	7.5	21.4	28.9	45.4	1015	16.6	81	14.0	12.4	140	540	1.94	1.41	110	170
	0.20	9.7	22.2	33.3	6.8	1317	9.8	83	13.6	11.7	60	760	1.31	0.84	30	110
E	0.54	22.4	35.3	49.6	11.2	3083	3.6	127	15.6	13.6	100	1630	1.59	1.16	80	270
	0.45	18.1	36.3	42.4	5.6	2446	10.6	72	14.8	11.5	50	1120	1.26	0.89	40	190
F	0.58	16.7	49.0	58.4	10.6	2320	15.7	131	14.1	11.0	130	1210	1.61	0.93	50	200
	0.40	21.9	55.0	69.9	4.1	3088	10.5	171	12.2	9.4	160	1350	1.56	0.89	70	240
Mean	0.43	17.4	32.2	44.1	11.4	2377	12.5	104	14.0	11.3	165	1195	1.56	1.07	63	207
SE	0.04	2.5	4.5	6.0	2.7	351	1.2	13	0.3	0.4	6.0	162	0.07	0.07	7	22
P			<0.005						<0.005				<0.005			

to experiment. Clear-cut increases in inulin clearance (C_{In}) were observed in 12 of the 15 experiments resulting in an average increase for all the experiments that was significant ($P < 0.005$). There were increases in the excretion of all electrolytes in each experiment. P_{Ca} fell in 14 of the 15 experiments. In 10 studies, it decreased by 2 mg% or more and in the remaining 4, by 1 mg% or more. The mean decrease for all experiments was 2.7 mg% which represented a 19.3% change from control. This decrease and the decrease in P_{Mg} were significant ($P < 0.005$).

Chronic experiments. Marked differences in susceptibility to the hypercalcemic effects of PTH were noted in the 10 dogs studied. Most animals became ill during the study with varying degrees of apathy, anorexia, vomiting and weight loss. Of the original 10 animals, 3 expired unexpectedly before the desired level of hypercalcemia could be induced. One of these died of apparent gastrointestinal bleeding, and one was subsequently noted to have had a SUN of 137 mg% shortly before death. A fourth rapidly developed hypercalcemia ($P_{Ca} = 17$ mg%) and died on the fifth day of PTH administration before furosemide was commenced. A fifth died during the period of furosemide administration with a SUN of 139 mg% before recovery periods could be obtained. This left 5 dogs in which complete studies were obtained.

Data from one such complete study are presented in Fig. 1. During Days 1 to 5, control values are shown for P_{Ca} and 24 hr excretion rates for urine sodium and calcium. Urine volumes ranged between 500 and 750 ml/24 hr and sodium excretion between 15 and 35 mEq/day. P_{Ca} was 10.1 mg%. On Day 5, PTH was commenced and the dose was adjusted to achieve a stable level of hypercalcemia which in this instance was between 14 and 15.5 mg%. During this 8-day period of PTH injection, urine volume gradually increased to 2.3 liters/24 hr and calcium excretion to 390 mg/day. Sodium excretion did not increase. Furosemide was then given in a

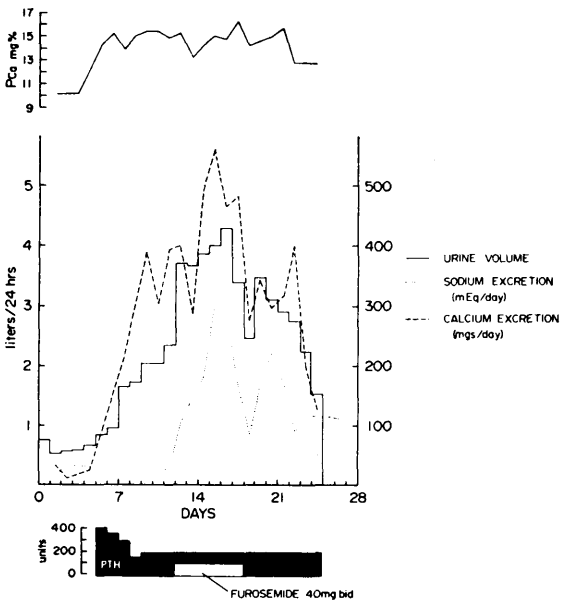

FIG. 1. Study showing effects of chronic furosemide administration on hypercalcemic dog.

dose of 40 mg (2.5 mg/kg) intramuscularly twice daily for 6 days. This resulted in further increases in calcium excretion but there was no clear-cut decrease of P_{Ca} during furosemide administration. In the postfurosemide recovery period, urine output and sodium and calcium excretion gradually fell and approached control levels. During the last 3 days of this period, P_{Ca} fell to 12.3 mg%. This may have been due to the development of PTH resistance or renal insufficiency with secondary depression of calcium.

This animal weighed 20.5 kg before PTH was begun. By Day 12, before furosemide was given, its weight had fallen to its lowest level of 18.2 kg. SUN rose from normal to 29 mg% on Day 12 and reached a maximum of 59 mg% on Day 16.

Mean data from the 5 chronic studies are shown in Figs. 2 and 3. PTH injection was associated with increases in urine output, P_{Ca}, P_{Mg} and calcium excretion. During furosemide administration, there was a striking diuresis, natriuresis and calciuresis but no significant change in potassium or magnesium excretion. Despite the statistically significant calciuretic effect, however, P_{Ca} remained unchanged. Finally, when furosemide was withdrawn during the recovery period, P_{Ca} did not change significantly.

As noted earlier, these animals lost weight and developed varying degrees of nitrogen retention. Weight loss in these 5 animals ranged between 11.2 to 30% of control with a mean loss of 20.1%. Azotemia occurred during the period of PTH administration and hypercalcemia with SUN ranging between 29 and 103 mg% (mean = 52 mg%). There was a further increase when furosemide was superimposed (mean = 80 mg%) with a tendency to fall during the recovery phase (mean = 58 mg%).

Discussion. The results of our acute experiments show that in the hypercalcemic dog, as in man (5), furosemide in large intravenous doses is effective in augmenting calcium excretion and significantly lowering P_{Ca} provided extracellular fluid volume is maintained during the course of the diuresis. The massive natriuresis induced by furosemide was accompanied on the average by a tenfold increase in calcium excretion as well as smaller increases in potassium and magnesium excretion, effects which are qualitatively similar to those observed in normal animals. In addition, under the conditions of our experiments, furosemide was also effective in lowering P_{Mg}.

Micropuncture studies on the mammalian

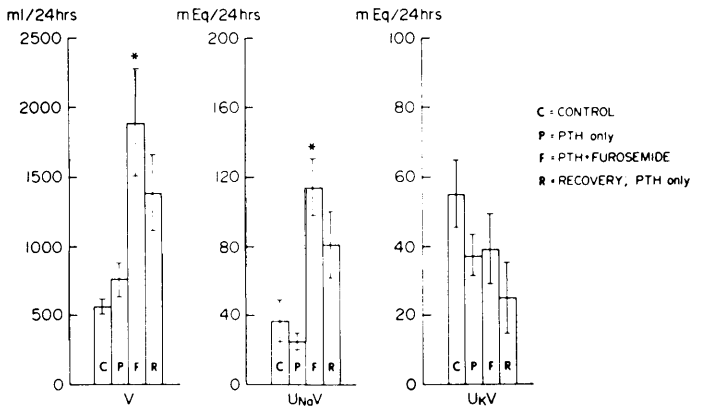

FIG. 2. Results of daily urine volume, sodium excretion and potassium excretion in the chronic studies. Values represent means; brackets, standard errors. (*) Significant differences between period F and period P ($P < 0.005$).

nephron have shown that filtered calcium is extensively reabsorbed in the proximal tubule and loop of Henle (8, 9). In the latter study, as much as 20 to 25% of filtered calcium has been shown to be reabsorbed in the loop of Henle where furosemide exerts a major natriuretic effect (10). Indirect studies have shown that a major portion of furosemide calciuretic effect is produced by inhibition of calcium reabsorption at this site (3). In addition, there is evidence from micropuncture studies in the rat that furosemide exerts a natriuretic effect on the proximal tubule (11). Whether it also inhibits calcium reabsorption at this site is uncertain (12).

Another mechanism of action pertinent to our results is the ability of furosemide to increase renal hemodynamics (13). Although renal blood flow was not measured in our acute studies, a 37% increase in GFR was observed. The combination of increased filtered load and depressed tubular reabsorptive capacity for calcium proved highly effective in lowering plasma levels. These same

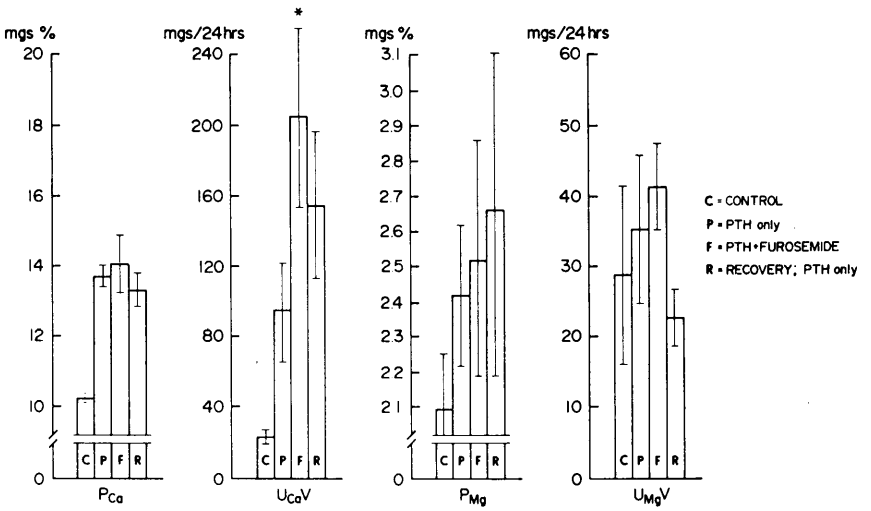

FIG. 3. Results of plasma calcium, calcium excretion, plasma magnesium and magnesium excretion in the chronic studies. Values represent means; brackets, standard errors. (*) Significant differences between period F and P ($P < 0.01$).

considerations also apply to magnesium.

In addition to the above, it is also possible that some of the calciuresis may have been due to increases in ECF volume secondary to the cumulative effect of Infusion I (1.7 ml/min) over the time course of the experiments (14). It is unlikely that this significantly augmented calcium excretion under our experimental conditions since large saline loads are required for this to occur. From an empirical standpoint, however, this effect could be carefully exploited once the furosemide-induced diuresis is underway.

In our chronic studies, furosemide significantly augmented calcium excretion but failed to lower P_{Ca}. The most striking difference between the two studies is the magnitude of the calciuresis. The average calcium excretion during furosemide administration was 205 mg/24 hr in the chronic studies. In contrast, calcium excretion in the acute studies averaged 1195 μg/min or 71.7 mg/hr. If this is translated to a 24 hr basis, calcium excretion would average 1720 mg, which represents an eightfold increase over that observed in the chronic experiments.

There are several explanations for the lesser calciuresis observed in the chronic studies. One possibility is that the animals in the latter group may have developed greater renal impairment than the others which blunted their calciuretic response. However, since furosemide is effective even in moderately advanced renal insufficiency, it is unlikely that quantitative differences in renal impairment constitute an adequate explanation. Much more probable are the differences in experimental design between the two groups. In the acute studies, furosemide was administered continuously with precise regulation of extracellular fluid volume over the 2-hr postfurosemide observation period. In contrast, furosemide was injected intermittently at 12-hr intervals in the chronic dogs while salt and water balance was corrected once daily. This could have resulted in intermittent dehydration which could then result in secondary decreases in calcium excretion in the interval between injections (15). Finally, plasma electrolytes were sampled during the immediate postfurosemide period in the acute studies and 12 hr after the last dose of furosemide in the chronic. It is therefore possible that we missed transient postinjection decreases in P_{Ca} even in this group.

These studies indicate that furosemide can effectively lower elevated levels of P_{Ca} provided certain conditions are met. In the presence of renal impairment it is necessary to use doses large enough to be calciuretic and dehydration must be avoided by meticulous control of salt and water balance during the course of the diuresis. It is also apparent that careful attention must also be given to potassium and magnesium balance to prevent concurrent depletion of these electrolytes. It would therefore appear that the greatest potential usefulness of furosemide is for the acute reduction of P_{Ca} pending the institution of definitive or long acting therapy.

Summary. Hypercalcemia was induced in dogs by the administration of calciferol or parathyroid hormone. When plasma calcium (P_{Ca}) levels reached 13–15 mg%, one of two protocols were commenced. In one, furosemide was administered acutely (5 mg/kg) in the setting of a renal clearance study and salt and water balance were meticulously controlled during the course of the diuresis. In the other, furosemide was given chronically (2.5–15 mg/kg) over several days and sodium balance was adjusted on a daily basis. Sodium, potassium, calcium and magnesium were measured in plasma and urine before and after furosemide administration. Although significant increases in calcium excretion were obseved in both acute and chronic studies, decreases in P_{Ca} occurred only in the acute studies. Our findings indicate that furosemide can effectively lower elevated levels of P_{Ca} by a combination of increased filtration and depressed tubular reabsorption of calcium provided depletion of extracellular fluid volume is prevented during the course of the diuresis.

1. Demartini, E. F., Briscoe, A. M., and Ragan, C., Proc. Soc. Exp. Biol. Med. **124**, 320 (1967).

2. Duarte, C. G., Metabolism **17**, 867 (1968).

3. Antoniou, L. D., Eisner, G. M., Slotkoff, G. M., and Lillienfield, L. S., J. Lab. Clin. Med. **74**, 410 (1969).

4. Eknoyan, G., Suki, W. N., and Martinez-Maldonado, M., J. Lab. Clin. Med. **76**, 257 (1970).

5. Suki, W. N., Yium, J. J., VonMinden, M., Saller-Herbert, C., Eknoyan, G., and Martinez-Maldonado, M., N. Engl. J. Med. **283**, 836 (1970).

6. Reimold, E. W., Metabolism **21**, 593 (1972).

7. Edwards, Allen L., "Statistical Methods," 2nd ed. Holt, Rinehart and Winston, New York (1967).

8. Duarte, C. G., and Watson, J. F., Amer. J. Physiol. **212**, 1355 (1967).

9. Lassiter, W. E., Gottschalk, C. W., and Mylle, M., Amer. J. Physiol. **204**, 771 (1963).

10. Suki, W., Rector, F. C., Jr., and Seldin, D. W., J. Clin. Invest. **44**, 1458 (1965).

11. Brenner, B. M., Keimowitz, R. I., Wright, F. S., and Berliner, R. W., J. Clin. Invest. **48**, 290 (1969).

12. Antoniou, L. D., Shalhoub, R. J., Gallagher, P., and O'Connell, J. M. B., Amer. J. Physiol. **220**, 816 (1971).

13. Ludens, J. H., Hook, J. B., Brody, M. J., and Williamson, H. E., J. Pharmacol. Exp. Ther. **163**, 456 (1968).

14. Massry, S. G., Coburn, J. W., Chapman, L. W., and Kleeman, C. R., Amer. J. Physiol. **213**, 1218 (1967).

15. Suki, W. N., Hull, A. R., Rector, F. C., Jr., and Seldin, D. W., J. Clin. Invest. **46**, 1121 (1967).

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