The effect of fluorocitrate on urinary calcium and citrate excretion

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Summary. The renal handling of calcium and citrate was studied in dogs after the administration of fluorocitrate. The drug produced a significant increase in urinary calcium and citrate excretion. Net renal secretion of citrate occurred during the infusion of fluorocitrate since citrate clearances exceeded the glomerular filtration rate.

The influence of citrate on renal calcium handling has not been fully evaluated. An increase in both calcium and citrate excretion follows infusion of citrate[2], or crude parathyroid hormone extract[3]. On the other hand, a dissociation between urinary calcium and citrate may occur after the administration of estrogen[4] and alkali[5]. Karam et al.[6] demonstrated in the rat that a decrease in renal tissue citrate produced by growth hormone was associated with an increase in urinary calcium excretion while increases in tissue citrate induced by fluoroacetate, which after conversion to fluorocitrate causes citrate accumulation by inhibiting aconitase[7], had the opposite effect. These authors postulated that intracellular rather than urinary citrate is a most important factor regulating urinary calcium excretion. In the present study, we have evaluated the renal handling of calcium and citrate in dogs after the i.v. administration of fluorocitrate.

Materials and methods. The studies were performed in 5 acidotic and 5 alkalotic female mongrel dogs (16–19 kg). Acidosis or alkalosis was induced by the daily administration of 10 g of ammonium chloride or sodium bicarbonate, respectively, for 5 days. On the sixth day, under pentobarbital anesthesia, 1 ureter and the ipsilateral renal vein were catheterized through a ventral incision. A catheter was placed in a femoral artery for blood sampling and monitoring blood pressure. A piece of cortex from the contralateral kidney was taken and frozen immediately in liquid nitrogen. This specimen was used for the determination of tissue citrate concentration. Following closure of the abdomen, a 60-min equilibration period was allowed. After a priming dose of 0.5 μCi/kg of ^{125}I-iodothalamate, a sustaining infusion of 0.15 μCi/min was then administered i.v. for glomerular filtration rate (GFR) and renal plasma flow (RPF) determination. 2 control 20-min urine collections were obtained while normal saline was infused at a rate of 0.75 ml/min. Mid-period samples of arterial and renal venous blood were drawn for measurement of pH, calcium, ^{125}I-iodothalamate and citrate. Urine was analyzed for sodium in addition to the above determinations. After the control period, a priming dose of 0.01 mmoles/kg of b.wt of fluorocitrate followed by a sustaining infusion of 6 μmoles/min was administered. After 30 min of equilibration, 3 further urine collections were performed. At the end of the experiment, tissue levels of citrate were measured.

Citrate was determined by the method of Moellering and Gruber[8]. Fluorocitrate did not interfere with the citrate determination. Calcium levels were measured by atomic absorption spectrophotometry and sodium by flame photometry. The GFR and RPF were measured according to the method of Elwood and Sigman[9].

Results and discussion. A representative experiment in an acidotic animal is illustrated in figure 1. During fluorocitrate administration, no significant changes were noted in arterial pH, urinary sodium excretion or GFR. As expected, arterial citrate levels increased slightly while calcium levels decreased. Urinary citrate excretion increased from 0.05 to 9 μmoles/min and calcium excretion increased from 0.06 to 0.173 mg/min. Tissue citrate rose from 0.101 to 3.99 μmoles/g of wet tissue. During alkalosis, the results were similar except that urinary citrate excretion was higher and calcium excretion lower than in acidotic animals. In all experiments, fluorocitrate increased urinary citrate to values that exceeded the quantity filtered. In acidotic animals (figure 2) mean baseline urinary calcium excretion was 83 ± 18 μg/min (SEM) and the values increased to 166 ± 5 μg/min 90 min after beginning the infusion of fluorocitrate ($p < 0.05$; paired t-test). During alkalosis, urinary calcium increased from 43 ± 10 μg/min 144 ± 10 μg/min after fluorocitrate

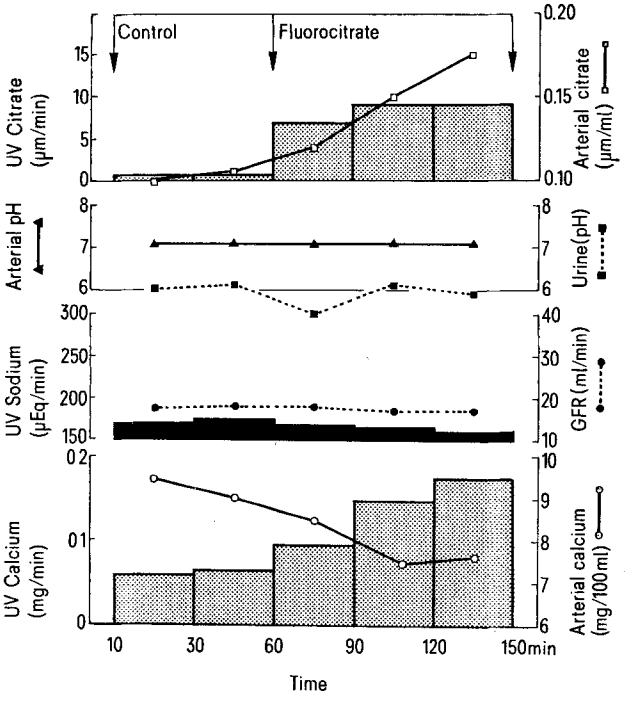

Fig. 1. Effect of fluorocitrate on renal handling on calcium and citrate during chronic metabolic acidosis.

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($p < 0.01$). Urinary calcium excretion remained unchanged or decreased in acidotic or alkalotic animals that did not receive fluorocitrate.

These studies demonstrate that administration of fluorocitrate, an aconitase inhibitor known to increase blood and tissue citrate content by inhibiting the conversion of citrate to isocitrate[7], is associated with an increased urinary calcium excretion. In contrast to the present study, Karam et al.[6] reported that fluoroacetate administration was associated with decreased urinary calcium excretion. These conflicting results, however, may be related to a divergent action of fluoroacetate and fluorocitrate as inhibitors of metabolism, since fluorocitrate, unlike fluoroacetate, causes striking increases in urinary citrate excretion despite similarly elevated blood and tissue citrate levels[10].

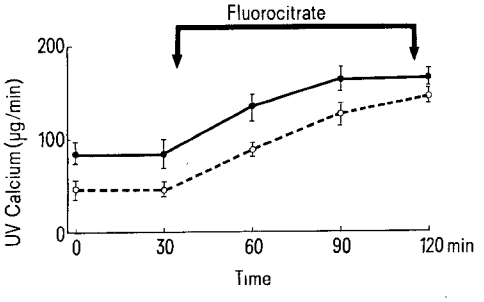

Fig. 2. Urinary calcium excretion in 5 acidotic (closed circles) and 5 alkalotic (open circles) dogs before and during the infusion of fluorocitrate. The vertical bars represent SEM. In both groups of animals the increments at 60 and 90 min were statistically significant with the paired t-test (acidosis: $p < 0.05$; alkalosis: $p < 0.01$) urinary calcium excretion decreased or remained unchanged in control animals.

The hypocalcemia produced by fluorocitrate administration would have been expected to stimulate parathormone release. Although this hormone increases tissue and urinary citrate excretion recent studies indicate that it lowers urinary calcium probably by stimulating distal tubular reabsorption[11]. The hypercalciuria previously reported after administration of parathyroid hormone[3] is probably related to the fact that crude extracts increase the filtered load of calcium, thereby, obscuring enhanced fractional reabsorption[12]. 2 main conditions enhance urinary citrate excretion: alkalosis and the administration of substrates of the Kreb's cycle, including citrate, presumably by inhibition of tubular reabsorption of citrate[13]. In the present study, fluorocitrate increased the urinary excretion of citrate to exceed the quantity filtered. Although the method used to measure glomerular filtration rate slightly underestimates the inulin clearance[14], the error of the method is too small to alter our results. Net citrate secretion has not been reported before, except during the infusion of malate[13]. Kook and Lotspeich[15], however, found ^{14}C-citrate in the urine of dogs given labelled precursors in stop-flow experiments.

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Arterial and mixed venous blood gases following DNP infusions in rabbits

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Summary. Hypocapnia and respiratory alkalosis was found in arterial and mixed venous blood after i.v. administration of DNP. Stimulation of ventilation resulted in steady P_aO_2, when PvO_2 decreased, and seemed to be independent on CO_2.

Ramsay[1] and Huch et al.[2] have shown an increase in body oxygen consumption following i.v. infusions of 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation in mitochondria. This effect was accompanied by an increase in lung ventilation, with arterial blood gases remaining close to control values.

The mechanism of respiratory response in the case of DNP-induced hypermetabolism is uncertain. The influence of the drug itself on respiration by direct central action, and/or action on carotid baro- and chemoreceptors, seems to be negligible, when compared with the total effect, as was shown by cross-perfusion experiments of Levine and Huckabee[3]. Therefore 2 possible sources of enhanced respiratory drive should be taken into account: tissue metaboreceptors, detecting rate of metabolism in muscles[4], and venous or pulmonary chemoreceptors, responding to changes in mixed venous blood composition[5]. In the present study, arterial and mixed venous pH, PCO_2 and PO_2 were measured in 6 rabbits (2.5–3.5 kg b.wt), lightly anaesthetized with sodium pentobarbitone (45 mg/kg, small supplementary doses added when signs of arousal were observed), and treated with DNP. Up to 6 infusions of 5 mg/kg DNP (1% solution in 1.5% $NaHCO_3$) were performed every 15–20 min. Blood samples (arterial – from right femoral artery, and mixed venous – from right atrium via right jugular vein) were taken prior to each infusion and analyzed for pH, PO_2 and PCO_2 with the Radiometer BMS-3 blood analyzer.

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