ORGANIC ACID EXCRETION AFTER CALCIUM GLUCONATE INFUSIONS

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Infusions of calcium gluconate are now often used in the investigation of skeletal disease. Nordin and Fraser (1956) found that osteomalacic patients retained an abnormally large proportion of the infused calcium in bone, with a corresponding reduced excretion of calcium in the urine. The hypercalcaemia after the infusion causes a fall in urinary phosphate clearance and rate of phosphate excretion thought to be due to a temporary reduced output of endogenous parathyroid hormone (Nordin and Fraser, 1954). Calcium infusions also cause diuresis with an increased urinary loss of sodium, potassium, and chloride (Levitt, Halpern, Polimeros, Sweet, and Gribetz, 1958; Freedman, Moulton, and Spencer, 1958). Freedman *et al.* (1958) report "a three-fold rise in excretion of undetermined anion which was possibly due to an increase in citrate excretion." The observations reported in this paper were designed to investigate this "undetermined anion" in more detail. It is shown that after calcium gluconate infusions the level of urinary organic acid rises appreciably, and is, as might be expected, chiefly gluconate. Urinary citrate is usually somewhat increased, but evidence is given that this is secondary to the rise of urinary calcium.

Methods

The infusions of calcium gluconate were carried out in the morning between 8 a.m. and 1 p.m. A basal specimen of urine was collected in the 12 hours immediately preceding the infusion. One hundred millilitres of 10% calcium gluconate added to 500 ml. of 5% glucose solution were infused at a steady rate over a five-hour period. Separate timed urine specimens were collected for the next 24 hours. No attempt was made to obtain urine samples at fixed times, as the infusions cause a variable and unpredictable diuresis, and it was desired to avoid collection errors as far as possible. All the samples were analysed for calcium, citrate, and total organic acid. The specimens containing most organic acid were also analysed for keto-acids, and the organic acids were examined qualitatively by paper chromatography.

The results are reported of 10 infusions classified as follows: (1) five infusions in healthy adult subjects in normal acid-base balance; (2) two infusions in healthy adult subjects made mildly acidotic by the ingestion of ammonium chloride, 2 g. four times daily for the two days preceding the infusion and the day of the infusion; (3) two infusions in adults suffering from severe osteomalacia due to idiopathic steatorrhoea; and (4) one infusion in one of the osteomalacic patients after four weeks' treatment with large doses of "calciferol" (1 m. units daily), resulting in considerable biochemical and radiological improvement.

Chemical Methods

Urinary Calcium.—The method of Shohl and Pedley (1922) was used.

Urinary Titratable Organic Acid.—A modification by Palmer (1926) of the original titration method of Van Slyke and Palmer (1920) was chosen.

Urinary Citrate.—The method of McArdle (1955) was used.

Urinary Keto-acids.—The method of McArdle (1957) was used.

Chromatography of Urinary Organic Acids.—Aliquots of the control and experimental urines were evaporated to dryness under reduced pressure. The residue was dissolved in a 1:1 mixture of methyl alcohol and water and chromatographed on Whatman No. 1 paper using three different solvent systems (one-way chromatography), namely, (1) butanol-acetic acid-water (120:30:50), (2) methanol-ethanol-N ammonia (45:45:10), (3) isopropanol-pyridine-water (120:40:40). The location reagents were silver nitrate and lead tetra-acetate.

Results

The changes produced in the excretion of calcium, citrate, and organic acid are shown in Figs. 1–3. Fig. 1 gives the effect on calcium output. There was no significant difference in calcium excretion in the normal and acidotic subjects. An average of 45% (range 39%–52%) of the infused calcium appeared in the urine. Calcium excretion was highest just after the end

FIG. 1.—Calcium excretion after an infusion of calcium gluconate in normal subjects, two osteomalacic patients, and acidotic normal subjects. Calcium excretion is unaffected by acidosis but is greatly reduced in the osteomalacic patients. The continuous lines give the mean excretion in normal and acidotic subjects and in the osteomalacic patients respectively.

of the infusion and had returned to basal levels within 20 hours. Both the basal calcium excretion and the amount excreted after the infusion were abnormally low in the osteomalacic patients, a mean of 10% (range 8%–12%) of the injected calcium being excreted. These values agree with the more extensive observations of Nordin and Fraser (1956), who reported a range of 33% to 53% calcium excreted in normal subjects, and less than 27% in cases of osteomalacia. To simplify the figure, the results obtained in the osteomalacic patient after treatment are not plotted in Fig. 1. The basal value of urinary calcium had not increased, but excretion of infused calcium rose from 8% to 22%.

Changes in total urinary organic acid are given in Fig. 2. The basal excretion varied from 20 to 65 $\mu\text{Eq./min.}$, which is a larger range than that reported in normal subjects by Van Slyke and Palmer (1920) of 28 to 52 $\mu\text{Eq./min.}$ The lowest values were in the acidotic subjects, in whom citric acid and keto-acid excretion is usually

reduced (Evans, MacIntyre, Macpherson, and Milne, 1957). After the infusion, urinary organic acid levels rose in all subjects, there being no significant difference between the four groups. Excretion was maximal immediately after the end of the infusion and had fallen to basal levels within 20 hours. The chromatographic separation showed that there was a slight increase of citric acid, but the main change was the appearance of a large spot with R_f values identical with those of pure gluconic acid (R_f values 0.03, 0.67, and 0.31 in systems 1, 2, and 3). The infusion did not affect the excretion of either α -ketoglutaric acid or pyruvic acid. If it is assumed that gluconate excretion can be estimated by the increase in total urinary organic acid less the rise in urinary citrate, the average urinary excretion of infused gluconate was 72% (range 64%–82%). Stetten and Topper (1953) found that after injection of gluconate labelled with ^{14}C in rats, 60%–85% was excreted as gluconate in the urine, the remainder being metabolized.

FIG. 2.—Excretion of titratable organic acid by the Van Slyke and Palmer method after an infusion of calcium gluconate. There is a considerable increase of urinary organic acid after the infusion with no significant difference in normal subjects, acidotic subjects, and cases of osteomalacia. The continuous line gives the mean of all observations.

Figs. 3 and 4 show the effect of the infusion on citrate excretion. The basal excretion varied from 0.8 to 1.8 $\mu\text{mol.}/\text{min.}$ in the normal subjects, but was less both in the acidotic subjects and in the untreated cases of osteomalacia. Östberg (1931) gave the normal range of citrate excretion in adults as 0.7 to 3.6 $\mu\text{mol.}/\text{min.}$ In the treated case of osteomalacia, the basal urinary citrate output had risen to 2.5 $\mu\text{mol.}/\text{min.}$ Calcium gluconate infusion had no effect on citrate excretion in the acidotic subjects and in the untreated cases of osteomalacia. In the normal subjects, citrate output increased in parallel with urinary calcium (Fig. 4), the maximum observed output being 5.0 $\mu\text{mol.}/\text{min.}$, which is slightly above the normal range. In the treated case of osteomalacia, there was a similar increase in citrate to a maximum excretion rate of 5.9 $\mu\text{mol.}/\text{min.}$ Fig. 4 shows that in the normal subjects there was a positive correlation between urinary citrate and calcium ($r = +0.81$; $P < 0.001$). The mean increase of urinary citrate was 1 $\mu\text{mol.}/\text{min.}$ for every 20 $\mu\text{Eq.}/\text{min.}$ rise in urinary calcium. Since the formula of the calcium citrate chelation

complex (Hastings, McLean, Eichelberger, Hall, and Da Costa, 1934) is $(\text{CaCit})^-$, this is only one-tenth the expected rise if all the excess calcium were excreted as chelate.

Discussion

Infusions of calcium gluconate result in complex metabolic effects, which may be classified as follows:

(a) An increase of plasma and urinary calcium (Nordin and Fraser, 1956), which effect is reduced in osteomalacia where calcium is retained by combination with osteoid matrix; (b) a decrease of urinary phosphate and diminished phosphate clearance (Nordin and Fraser, 1954); (c) osmotic diuresis with increased excretion of sodium, chloride, and potassium (Freedman *et al.*, 1958; Levitt *et al.*, 1958); (d) increased organic acid excretion. Excretion of gluconic acid is obviously directly related to the gluconate infusion. This excess urinary anion is excreted as sodium and potassium salts, and partially accounts for the increased loss of these cations after the infusion. The present observations suggest that any increase

FIG. 3.—Citrate excretion after a calcium gluconate infusion in normal subjects, two osteomalacic patients, and acidotic subjects. The continuous line gives the mean excretion in the normal subjects. Citrate output is abnormally low both in acidosis and in the cases of osteomalacia.

FIG. 4.—Relationship between urinary citrate and urinary calcium after calcium gluconate infusions. There is a positive and significant correlation between urinary calcium and citrate in both normal subjects and in a case of osteomalacia treated with calciferol. In acidotic subjects and in cases of untreated osteomalacia, urinary citrate is abnormally low. The straight line gives the calculated regression line in normal subjects.

of urinary citrate in normal subjects (Figs. 3 and 4) is directly related to the hypercalciuria. In the osteomalacic patients there was only a slight rise of urinary calcium and no increase in urinary citrate. Acidosis always reduces citrate excretion (Östberg, 1931; Clarke, Evans, MacIntyre, and Milne, 1955) and here the influence of abnormal acid-base balance overwhelmed that of the hypercalciuria, there being no increase of urinary citrate with rise of urinary calcium.

Shorr, Almy, Sloan, Taussky, and Toscani (1942) have previously reported a positive correlation between urinary calcium and citrate when excretion is modified by changes in calcium intake. Their data from a case of primary hyperparathyroidism are given in detail, the correlation coefficient between urinary calcium and citrate being $+0.79$. The quoted values of urinary citrate were higher than in our normal subjects, but comparable with the treated case of osteomalacia (Fig. 4). The most probable explanation of this correlation is that the calcium citrate chelation complex is less completely reabsorbed by the renal tubules than

either calcium ions or free citrate. A rise of the urinary calcium level therefore tends to increase urinary citrate, and, vice versa, citrate infusions may cause a rise of urinary calcium (Chang and Freeman, 1950; Freeman and Chang, 1950).

Systemic acidosis enhances the renal tubular reabsorption of citrate filtered at the glomeruli, with a consequent reduction in the urinary citrate loss (Clarke *et al.*, 1955). The free citrate within the fluid in the tubular lumen falls, causing dissociation of the calcium citrate chelation complex. In this case urinary citrate may therefore be low despite hypercalciuria. The administration of physiological doses of vitamin D to normal or rachitic rats leads to an increased urinary output of citric acid (Bellin and Steenbock, 1952), and to higher concentrations of citric acid in the blood and the bones (Carlsson and Hollunger, 1954). Harrison and Harrison (1952) showed that serum and urinary citrate levels in rachitic infants were increased by vitamin D therapy. The present results suggest that the changes in urinary citrate may precede a rise of

FIG. 5.—Precipitates of calcium phosphate in alkaline urines. 5a.—After sodium bicarbonate and with increased urinary citrate. 5b.—After acetazolamide causing decreased urinary citrate. Passage of the large angular crystals after acetazolamide was accompanied by severe strangury. $\times 36$ in both cases.

the urinary calcium level. De Luca, Gran, and Steenbock (1957) have explained the action of vitamin D on citrate by showing an effect at the enzymatic level of metabolism. Homogenates of rat kidneys from animals receiving excess calciferol showed a 24% decrease in the rate of citrate oxidation by partial inhibition of the activity of iso-citrate dehydrogenase. Changes in urinary citrate due to calciferol may therefore be secondary to alteration of levels of renal tissue citrate.

The interrelations of urinary citrate and calcium may be of direct physiological importance in the prevention of nephrocalcinosis and the formation of renal calculi. Citrate is the most efficient natural calcium chelating agent in urine. Tricarboxylic acids chelate calcium more strongly than dicarboxylic or monocarboxylic acids (Joseph, 1946), and citric acid itself is more effective than the closely related tricarballic acid (Schubert and Lindenbaum, 1950). Urinary citrate has been reported to be low in cases of renal calculi (Kissin and Locks, 1941; Scott, Huggins, and Selman, 1943), but this may partly be due to bacterial destruction of urinary citrate from associated urinary infection (Conway, Maitland, and Rennie, 1949). Citrate excretion is high in alkalosis (Evans *et al.*, 1957), and therefore the tendency to precipitation of calcium phosphate in alkaline urine is reduced. Liability to nephrocalcinosis or stone formation would be expected in states of systemic acidosis with reduced urinary citrate accompanied by an abnormally alkaline urine. This combination occurs in renal tubular acidosis and after inhibition of renal carbonic anhydrase. Renal tubular acidosis is commonly associated with nephrocalcinosis. Efficient therapy by administration of alkali causes a rise of the previously low urinary citrate to normal levels (Bauld, MacDonald, and Hill, 1958). Prolonged treatment with acetazolamide greatly reduces urinary citrate (Clarke *et al.*, 1955), and may be complicated by renal stone formation (Gordon and Sheps, 1957), or ureteric obstruction from crystalluria (Yates-Bell, 1958). A more common and less serious toxic symptom in male patients is strangury from the presence of large crystals of calcium phosphate (Fig. 5). Urinary citrate both increases the solubility of calcium phosphate by chelation and prevents the formation of large angular crystals.

Calcium phosphate is therefore usually precipitated as a fine amorphous debris which causes no urethral irritation.

Summary

Infusions of calcium gluconate cause a considerable increase of urinary organic acid. This is chiefly due to excretion of the infused gluconate, an average of 72% being excreted in the urine.

In normal subjects there is also a smaller rise of urinary citrate which parallels the rise of urinary calcium. In acidotic subjects and in cases of untreated osteomalacia, basal excretion of citrate is low and there is little or no increase of urinary citrate after the infusion. In one case of osteomalacia, calciferol treatment caused a rise of urinary citrate before the urinary calcium level was significantly increased.

The implications of the urinary calcium and citrate interrelationship in the precipitation of calcium phosphate and development of nephrocalcinosis and renal calculus are discussed.

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