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Edwin E. Gordon

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EFFECT OF ACUTE METABOLIC ACIDOSIS AND ALKALOSIS ON ACETATE AND CITRATE METABOLISM IN THE RAT *

By EDWIN E. GORDON †

(From the Department of Medicine, Albert Einstein College of Medicine, Yeshiva University,
and the Bronx Municipal Hospital Center, New York, N. Y.)

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An enhanced urinary excretion of citrate after administration of alkali and a diminished citrate excretion after administration of ammonium chloride were first observed by Östberg in 1931 (1). More recently, it has been pointed out that the quantity of citrate excreted in the urine is not a direct consequence of acidification or alkalinization of the urine as believed by Östberg, but rather is related to systemic pH (2). Hypercitraturia is associated with alkalosis and hypocitraturia with acidosis. This correlation appears to be independent of the mechanism of production of the altered acid-base balance. Thus, urinary citrate is diminished in diabetic acidosis (1, 3, 4), as a result of the intracellular acidosis of potassium depletion (5, 6), and in the metabolic acidosis induced by acetazolamide (2, 7, 8), as well as by administration of NH_4Cl , CaCl_2 , and HCl . Increased excretion of citrate in the urine has been observed during respiratory alkalosis (4, 6, 9) as well as during metabolic alkalosis.

Two hypotheses have been proposed to explain this effect of altered acid-base balance on urinary citrate excretion. One hypothesis holds that alterations of pH influence the synthesis of citrate and its subsequent secretion by renal tubules (10). This implies that the intracellular pH of the renal tubular cell influences substrate metabolism in the Krebs citric acid cycle with subsequent transport of metabolites from the renal cell into the tubular urine. The second hypothesis, based on data obtained by conventional clearance techniques (11, 12), suggests that acidosis results in

an increased renal tubular reabsorption of citrate and alkalosis in a decreased tubular reabsorption of filtered citrate. This would implicate membrane transport as a major mechanism affected by pH changes.

It has previously been shown in this laboratory that the urinary citrate is at least in part derived from the blood citrate, since an appreciable portion of intravenously administered citrate- C^{14} appears unchanged in the urine (13). In the present investigations, a similar experimental approach employing radioactive tracer substances was used to study the mechanism by which acute alterations of acid-base balance influence urinary citrate excretion. These experiments support the second hypothesis, namely, that there is a pH effect on renal tubular reabsorption of filtered citrate. In addition, they provide information bearing on the origin and metabolic fate of the blood citrate in alkalosis and acidosis.

MATERIALS AND METHODS

Fed, female, Sprague-Dawley rats weighing approximately 200 g were used. HCl or Na_2CO_3 , 1.5 mmoles, was administered intragastrically in a volume of 3 ml; control animals received 3 ml of water. In all experiments, 0.3 ml of the radioactive compound was injected intravenously as a neutral solution in isotonic saline 30 minutes after administration of the test solution. Animals were restrained in wire cages, and precautions were taken to separate urine and feces during the 3-hour collection period. At the appropriate times, the animals were encouraged to empty their bladders by painful stimulation. The urine and washings were filtered and made up to volume before assay for C^{14} in urine, in urinary citrate, and for total citrate content. At the end of the collection period, the rats were anesthetized with ether, and blood was drawn from the abdominal aorta for determination of citrate concentration.

The pH of mixed tail blood was determined with a Radiometer pHM 4 meter and Astrup micro blood glass electrode. The citric acid-1,5- C^{14} was obtained from

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Tracerlab, Inc., and Na acetate-1-C¹⁴ from the New England Nuclear Corp.

The radioactivity of the urine and of the urinary citrate was determined by methods previously described with appropriate corrections for self-absorption and conversion of values to infinite thickness (13). Quantitative analysis of blood and urinary citrate was performed as previously described, by the technique of Elliott (14). For the determination of the specific radioactivity of the plasma citrate, the cells were separated from heparinized blood and the plasma proteins were precipitated by heating at acid pH; the supernatant solution was then subjected to ion-exchange chromatography on a Dowex-2 column in the formate form (15). The column eluate containing the organic acids was dried under reduced pressure at 40° C. An aqueous solution of the organic acids was prepared, and a sample was subjected to ascending paper chromatography on Whatman no. 1 paper in the II solvent system of Hartley and Lawson (16). Citric acid was then eluted from the paper, and samples were taken for plating and counting at infinite thinness in a Nuclear-Chicago gas-flow counter with Micromil window, and for citric acid analysis.

RESULTS

Effect of alkalosis and acidosis on fate of i.v.-administered citrate-C¹⁴. Intubation of the rat's stomach with a special needle¹ could usually be

¹ No. 71-042, Phipps and Bird, Inc.

accomplished in less than half a minute. Despite the ease and rapidity of the procedure, the pH of the blood fell as much as 0.1 U within 5 minutes regardless of the pH of the fluid administered (Figure 1). The blood pH subsequently rose to control levels in the animals that received water, to levels significantly above the control value in those that received alkali, and continued to fall during the observation period in the animals that received acid. There was a slightly greater and more prolonged elevation of the blood pH after 3 ml of 1.0 M Na₂CO₃ compared to that seen after 3 ml of 0.5 M Na₂CO₃.

The citrate concentration of the blood was unaltered by alkali or acid administration (Table I). Examination of the blood citrate concentration at 30 and at 60 minutes after administration of the test solution indicated that the values did not differ significantly among control, alkalotic, and acidotic rats. After 30 minutes the values were 31.8 ± 1.6, 31.0 ± 0.6, and 27.1 ± 1.0 μg per ml, and after 60 minutes they were 29.4 ± 1.8, 28.3 ± 1.3, and 27.1 ± 1.6 μg per ml for control, alkalotic, and acidotic rats, respectively. Urinary citrate increased in the alkalotic and decreased in the acidotic rat (p < .001, control against each

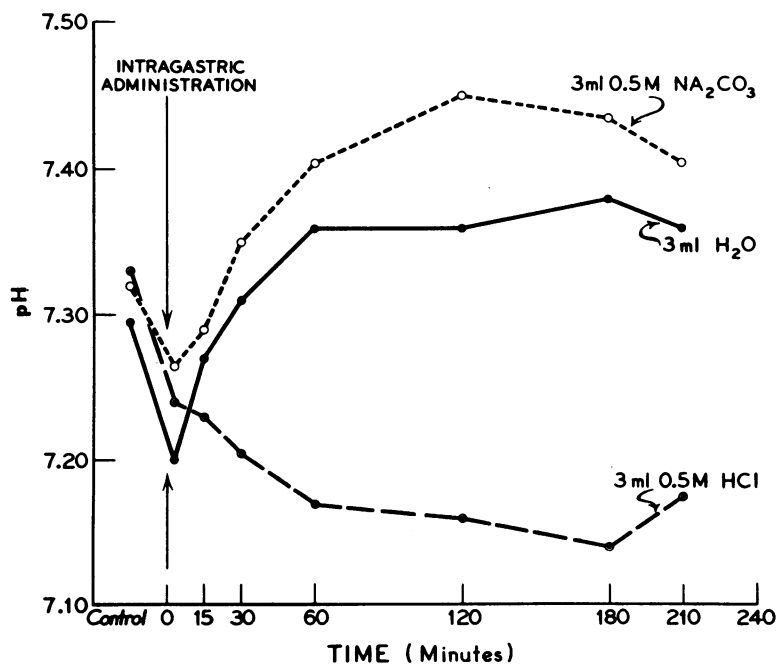


FIG. 1. BLOOD pH AFTER INTRAGASTRIC ADMINISTRATION OF WATER, ALKALI, AND ACID. Values for each treatment are the means of 3 rats.

TABLE I
Citrate concentration in blood and urine of normal, acidotic, and alkalotic rats *

| | Acidotic | Control | Alkalotic |
|---------------------------------|-------------------------|-------------------------|--------------------------|
| Blood citrate, $\mu\text{g/ml}$ | 25.0 \pm 1.6 [10] | 28.3 \pm 0.9 [7] | 27.4 \pm 0.9 [13] |
| Urine citrate, mg/3 hours | 0.49 \pm 0.15 [10] | 5.33 \pm 0.42 [10] | 10.31 \pm 0.47 [10] |

* Values are the means \pm standard error of the number of experiments indicated in brackets.

experimental group). The urine collected during the 3 hours after intravenous injection of 4 μg citrate- C^{14} (1.1×10^5 cpm) contained approximately 30 per cent of the administered C^{14} in control, 65 per cent in alkalotic, and 5 per cent in acidotic rats (Figure 2). Further analysis indicated that almost all of the C^{14} in the urine was present in the carboxyl groups of the urinary citrate. That is, the administered citrate was excreted in the urine unchanged, since the urinary citrate was labeled in the same positions as the administered radioactive citrate.

In other experiments, twice as much Na_2CO_3 (3.0 mmoles per rat) was administered. The values for blood citrate concentration, urinary citrate concentration, and percentage of counts in the urine and urinary citrate were indistinguishable from those seen after administration of 1.5 mmoles per rat.

A study of the rate of urinary excretion of injected citrate- C^{14} in control and alkalotic rats clearly indicated the rapidity with which the kid-

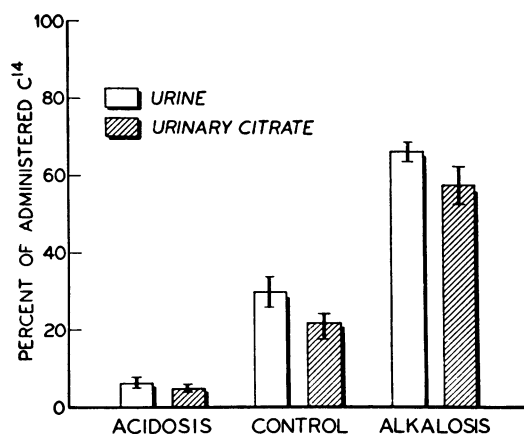


FIG. 2. URINARY C^{14} AFTER ADMINISTRATION OF CITRATE- C^{14} TO CONTROL, ACIDOTIC, AND ALKALOTIC RATS. Values are the means of 5 rats \pm standard error.

TABLE II
Rate of C^{14} appearance in urinary citrate of control and alkalotic rats after i.v. citrate- C^{14} *

| Time | Control | Alkalotic† |
|-----------------|---------|------------|
| <i>min</i> | | |
| 0-30 | 0.58 | 0.62 |
| 30-60 | 0.18 | 0.20 |
| 60-120 | 0.14 | 0.14 |
| 120-180 | 0.09 | 0.04 |
| Total (3 hours) | 1.00 | 1.00 |

* The value for each period is expressed as a fraction of the total citrate- C^{14} excreted in the urine during the 3-hour collection period. Three rats were used in each series.

† 1.5 mmoles Na_2CO_3 intragastrically 30 minutes before injection of citrate- C^{14} .

ney cleared the labeled plasma citrate (Table II). Of the total citrate- C^{14} excreted in the urine, more than half had appeared within 30 minutes and approximately three fourths had been cleared by the kidneys in the first hour in both groups of animals.

Effect of alkalosis and acidosis on fate of i.v.-administered acetate- C^{14} . Approximately 1 to 1.5 per cent of the C^{14} of 34 μg of intravenously administered Na acetate-1- C^{14} (7×10^6 cpm) was found in the urine of control animals during the 3-hour collection. In alkalotic rats about 5 per cent, and in acidotic rats about 0.2 per cent of the C^{14} was recovered in the urine (Figure 3). Despite the relatively small recoveries of C^{14} in the urine after acetate- C^{14} injection, the values for the experimental groups were significantly different from those of the control group ($p < .001$). Most of the radioactivity in the urine of alkalotic ani-

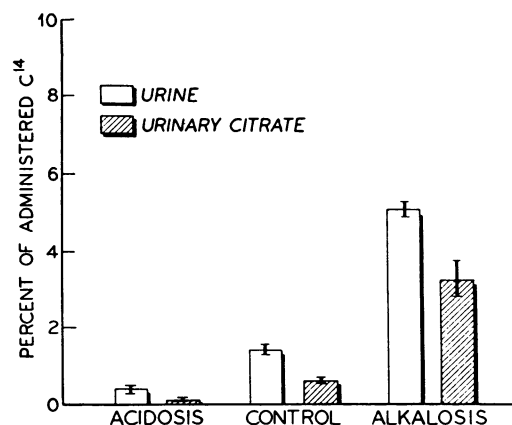


FIG. 3. URINARY C^{14} AFTER ADMINISTRATION OF ACETATE- C^{14} TO CONTROL, ACIDOTIC, AND ALKALOTIC RATS. Values are the means of 5 rats \pm standard error.

TABLE III
Rate of C^{14} appearance in urinary citrate of control and alkalotic rats after i.v. acetate- C^{14} *

| Time | Control | Alkalotic† |
|-----------------|---------|------------|
| <i>min</i> | | |
| 0-30 | 0.58 | 0.26 |
| 30-60 | 0.18 | 0.28 |
| 60-120 | 0.14 | 0.31 |
| 120-180 | 0.09 | 0.15 |
| Total (3 hours) | 1.00 | 1.00 |

* The value for each period is expressed as a fraction of the total citrate- C^{14} excreted in the urine during the 3-hour collection period. Three rats were used in each series.

† 1.5 mmoles Na_2CO_3 i.g. 30 minutes before injection of acetate- C^{14} .

mals was present in the urinary citrate; no attempt was made to assay the C^{14} content of urinary constituents other than citrate, but it seems likely that the undefined compounds containing the radioactivity were other organic intermediates or bicarbonate.

Administration of acetate- C^{14} to rats that had received twice as much alkali (3 mmoles per rat) resulted in essentially the same findings as those seen after the smaller amount.

The rate of acetate- C^{14} incorporation into the urinary citrate of control and alkalotic rats is indicated in Table III. The rate of appearance of the C^{14} into urinary citrate in control rats is the same as that seen after intravenous citrate- C^{14} administration. That is, of the total urinary citrate- C^{14} in the 3-hour collection period, more than one half is excreted in 30 minutes. In alkalotic rats, however, the rate of appearance was delayed. Citrate- C^{14} continued to appear in the urine in appreciable amounts throughout the collection period. These data provide evidence for a continuous synthesis of plasma and urine citrate; release of citrate from pools, such as are known to be present in bone (17), has not been ruled out.

Effect of alkalosis on synthesis of blood citrate in nephrectomized rats. In the nephrectomized preparation, a major organ of utilization and of

TABLE IV
Incorporation of acetate- C^{14} into the plasma citrate of nephrectomized rats

| | Control (5 Rats) | Alkalotic (4 Rats) |
|---------------------------------|------------------|--------------------|
| Plasma citrate, $\mu g/ml$ | 196 \pm 18 | 194 \pm 15 |
| Specific activity, cpm/ μg | 16.2 \pm 2.8 | 18.7 \pm 2.9 |

excretion of the plasma citrate is eliminated. Under these conditions, an effect of alkali administration on synthesis and release of citrate from extrarenal tissue would be accentuated. The values for blood citrate concentration and the specific activity of the plasma citrate 2.5 hours after oral administration of the test substance (3.5 hours after nephrectomy) and 30 minutes after 60 μc of Na acetate- C^{14} (200 μc per mg) are seen in Table IV. Although these data confirm the previous observations of blood citrate elevations in nephrectomized rats (18), they fail to reveal any difference between the nephrectomized rats that received water and those that received alkali. Neither the blood citrate concentration nor the specific activity of the plasma citrate differed in the two groups of animals. It therefore seems unlikely that acute alkali administration results in a significant extrarenal citrate synthesis or release.

DISCUSSION

The key role of citrate in the metabolic pathway leading to energy production, and the dramatic changes in urinary citrate excretion in acidosis and alkalosis naturally lead one to associate these events. The hypothesis that citrate metabolism in the renal tubular cell is closely related to urinary citrate excretion is particularly attractive because of the known avidity of the kidney for the blood citrate (19, 20) and because of the demonstrated rapid metabolism of citrate by kidney tissue (13). The present experiments conclusively demonstrate an effect of acidosis and alkalosis on renal clearance of plasma citrate. The data are consistent with the view that alkalosis results in a decreased tubular reabsorption of filtered citrate and acidosis in an increased tubular reabsorption of filtered citrate. These conclusions agree with interpretations based on conventional clearance methods (11, 12) and on stop-flow analysis (21). An effect of acute alterations of acid-base balance on tubular secretion of plasma citrate has not been ruled out.

It has been suggested (10) that alterations of pH influences synthesis and subsequent secretion of citrate by the renal tubules. Although hypercitraturia has been shown to occur in association with an increased citrate concentration in kidney tissue in such diverse conditions as alkalosis (22),

after sodium malate administration (23), and in fluorocitrate-poisoned rats (13), the increased urinary excretion need not be a consequence of the increased tissue concentration (13, 24). The data obtained in the present study suggest that tubular synthesis of citrate is not a major determinant of citrate excretion in the urine.

When acetate- C^{14} , a metabolic precursor of citrate, was administered, an increased incorporation of the C^{14} into urinary citrate was observed in alkalosis and a decreased incorporation in acidosis. The most likely interpretation of these data is an increased renal clearance of plasma citrate that had been labeled with C^{14} by condensation of the acetate- C^{14} with oxaloacetate in tissues. It is clear that labeled acetate is incorporated into plasma citrate (Table IV), and that this radioactively labeled citrate would be excreted by the kidneys in accordance with the demonstrated effect of pH alterations on the renal clearance of plasma citrate. It is of interest that tubular secretion of malate, another member of the tricarboxylic acid cycle, has been demonstrated (25, 26); alterations of acid-base balance do not appear to influence the urinary excretion of this intermediate (4).

The present studies have demonstrated an increased renal clearance of plasma citrate in the alkalotic rat without diminution of the concentration of citrate in the plasma. This finding suggests two possibilities, increased release of citrate into the blood, and decreased uptake and metabolism of plasma citrate by the tissues. The first appears to be remote in view of the present inability to demonstrate an increased incorporation of acetate- C^{14} into plasma citrate of alkalotic nephrectomized rats. The second possibility, diminished metabolism of blood citrate in the alkalotic rat, is supported by experiments with citrate- C^{14} . In the normal animal, approximately 55 per cent of an administered dose of citrate- C^{14} is found in the respiratory CO_2 in 3 hours (13). In the alkalotic animal, only 35 per cent at most could be metabolized, since 65 per cent of the administered citrate- C^{14} is excreted in the urine. In agreement with this hypothesis, Mårtensson (19) has provided data which suggest that metabolism of citrate by the kidneys increases in acidosis and decreases in alkalosis. Preliminary experiments in this laboratory indicate that citrate metabolism by

rat kidney slices is greater when they are incubated at pH 6.5 than at physiological or slightly alkaline pH (27). Clarification of this relationship between effects of altered pH on intracellular citrate metabolism and tubular reabsorption of citrate must await further experimentation.

SUMMARY

The mechanism of the altered urinary citrate excretion in acute metabolic alkalosis and in acute metabolic acidosis was studied in rats. Systemic pH influences the renal clearance of plasma citrate- C^{14} . The data are consistent with the mechanism of increased tubular reabsorption of filtered citrate in acidosis and decreased tubular reabsorption of filtered citrate in alkalosis.

Data have been presented which suggest that there is a continuous synthesis and release of citrate from extrarenal tissues, and that alkali administration does not influence this process.

The evidence for a relationship between citrate metabolism by the kidney and citrate excretion in the urine, under conditions of altered acid-base balance, has been summarized.

ACKNOWLEDGMENT

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ANNOUNCEMENT OF MEETINGS

THE AMERICAN FEDERATION FOR CLINICAL RESEARCH will hold its Twentieth Annual Meeting in Atlantic City, N. J., at the Casino Theatre on the Steel Pier on Sunday, April 28, 1963, at 9:00 a.m. Joint sectional meetings with The American Society for Clinical Investigation will be held on Sunday afternoon at Chalfonte-Haddon Hall, and additional meetings sponsored by The American Federation for Clinical Research will be held there on Sunday evening.

THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION, INC., will hold its Fifty-fifth Annual Meeting in Atlantic City, N. J., on Monday, April 29, at 9:00 a.m., at the Casino Theatre on the Steel Pier and in simultaneous programs sponsored with The American Federation for Clinical Research on Sunday afternoon, April 28, in Chalfonte-Haddon Hall.

THE ASSOCIATION OF AMERICAN PHYSICIANS will hold its Seventy-sixth Annual Meeting in Atlantic City, N. J., at the Casino Theatre on the Steel Pier on Tuesday, April 30, at 9:30 a.m., and in the Vernon Room, Chalfonte-Haddon Hall, on Wednesday, May 1, at 9:30 a.m.