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Tissue Citrate Levels and Citrate Utilization after Sodium Bicarbonate Administration.* (28647)

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Cells of the renal tubules normally reabsorb and oxidize nearly all of the citrate present in the glomerular filtrate(1). Metabolic alkalosis decreases citrate reabsorption and produces a large increase in the renal clearance of citrate(2). A rise in renal citrate concentration also occurs(3). The effect of metabolic alkalosis on citrate utilization by other organs has not been described. The specificity of the effect of bicarbonate on citrate utilization *in vivo* has been examined in the present study by comparing the effect of injections of sodium chloride or of sodium bicarbonate on (a) oxidation and excretion of intravenously administered, C-14 labeled citrate, and (b) tissue citrate levels in kidney, liver and heart.

Methods. Metabolism of citrate-1,5-C^{14}. Each of 8 male Sherman rats, previously fasted overnight, was anesthetized with ether and a polyethylene catheter tied into the bladder. The peritoneal incision was tightly sutured and sealed with collodion and the rat placed in a restraining cage. Ninety minutes after operation half the animals were injected intraperitoneally with 8 mM per kg of 0.1 M NaCl and the other half received the same amount of $NaHCO_3$. Thirty minutes later 2.5 μc of sodium citrate-1,5-C^{14} were injected into a tail vein. The rat was immediately placed in a metabolism jar and an oiled syringe attached to the bladder catheter. Dry air was passed through the metabolism jar and respiratory CO_2 was collected in 250 ml of 1 N KOH. CO_2 absorbing bottles were changed at intervals during a 2-hour period. The absorbed CO_2 was precipitated as the barium salt and radioactivity determined in a gas-flow proportional counter. At the end of the experiment the animal was anesthetized and blood drawn by heart

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puncture. pH was determined anaerobically on whole blood at room temperature; readings were corrected to 38°C by a factor of –0.014 units per degree. Because the animals were anesthetized for bleeding, pH values are only rough approximations of true systemic pH prior to anesthesia. The remainder of the blood was immediately centrifuged under oil at 2°C. Plasma CO_2 content was determined by the method of Van Slyke and Neill. Citric acid was estimated in boiled urine samples and trichloroacetic acid extracts of plasma by McArdle's modification of the pentabromacetone method(4).

Radioactivity of urinary bicarbonate and CO_2 was estimated by acidifying an aliquot of urine with an equal volume of 1 N sulfuric acid in a Warburg flask and absorbing the released CO_2 in 5 N sodium hydroxide in the center well; barium carbonate precipitates of this solution contained less than 0.1% of the injected radioactivity in each instance. To estimate the radioactivity of urinary citrate, 1 ml of the acidified urine was mixed with 4 g of silicic acid and placed in a column 18 mm in diameter. Organic acids were eluted with 200 ml of ether and the ether evaporated over 2 N sulfuric acid. An aliquot of the sulfuric acid solution was placed in a Warburg flask and the citric acid decarboxylated with ceric sulfate(5); CO_2 was collected in 5 N sodium hydroxide in the center well and the radioactivity determined on barium carbonate precipitates. Because ceric sulfate decarboxylation is not specific for citric acid, in one instance citric acid was isolated chromatographically from a urine sample and the radioactivity determined. Urine was passed through an anion exchange column and the organic acids eluted with formic acid(6). The eluate was evaporated to dryness, the residue dissolved in a small amount of water and placed on a strip of Whatman No. 1 filter paper. Descending chromatography was employed with a solvent system of n-butanol, formic acid, water in proportions of 10:2:15. The dried chromatogram was passed through a strip scanner which showed all the discernible radioactivity present in the citrate peak except for a small amount which remained at the origin. The citrate peak was cut out, the citrate eluted and decarboxylated with ceric sulfate; this fraction contained 83% of the radioactivity obtained after decarboxylation of the ether eluate from the silicic acid column.

Tissue citrate levels. After fasting overnight, six 200 to 300 g male Sherman rats were given an intraperitoneal injection of 8 mM per kg of 0.1 M NaCl. Six other rats in the same weight range were given the same dose of 0.1 M $NaHCO_3$. Two hours later the animals were killed by decapitation, and the kidneys, liver and heart were rapidly removed, blotted free of blood and frozen in liquid nitrogen. Frozen organs were ground to a fine powder in a chilled iron mortar and 10% trichloroacetic acid was added to weighed quantities of the powder. Citric acid was estimated in aliquots of the trichloroacetic acid extracts by McArdle's method(4).

Results. Metabolism of citrate-1,5-C^{14}. The effects of sodium chloride and sodium bicarbonate on the metabolism and urinary excretion of C^{14}-labeled citrate are shown in Fig. 1 and Table I. Urinary citrate and urinary C^{14}-labeled organic acid were several times greater after sodium bicarbonate than after sodium chloride, a result in agreement with that reported by Gordon(7). A decrease of about 10% in the recovery of respiratory $C^{14}O_2$ was present after sodium bicarbonate treatment. The magnitude of this effect is similar to the increase in the amount of organic acid-C^{14} in the urine of the bicarbonate treated animals. Plasma citrate increased significantly after $NaHCO_3$ but the percentage change between the two groups was small.

Tissue citrate levels. The tissue citrate levels in kidney, liver and heart after sodium chloride or sodium bicarbonate administration are shown in Fig. 2. There was a rise of about 3-fold in renal tissue citrate ($p<0.001$). Liver citrate increased by only one-third, but significantly ($0.005<p<0.01$). Heart citrate remained unchanged ($p>0.05$).

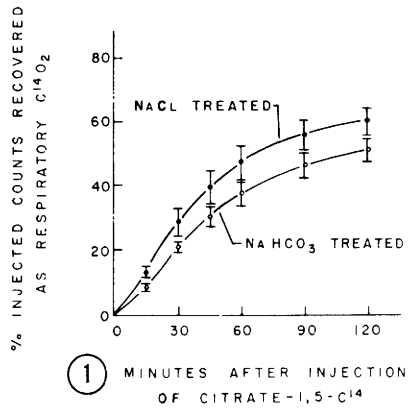
Discussion. The results confirm that urinary citrate and renal tissue citrate increase several-fold after sodium bicarbonate administration. The rise in renal tissue citrate seems too large to be accounted for by an increase in tubular fluid citrate alone. This sug-

TABLE I. Plasma and Urine Values After Injection of Citrate-1,5-C^{14} in Rats Previously Given NaCl or $NaHCO_3$ Intraperitoneally.

Substance inj	Plasma citrate (μM/ml)	Plasma pH	Plasma CO_2 content (μM/ml)	Urine citrate (μM/ml)	Urine citrate (μM/hr)	Urine organic acid-C^{14} (% inj counts)
NaCl	.19 ± .025	7.30 ± .04	24.4 ± 3.8	.33 ± .21	.72 ± .38	.98 ± .03
$NaHCO_3$	.25 ± .026	7.45 ± .08	39.0 ± 1.4	1.32 ± .15	3.80 ± 1.18	7.2 ± 2.8

Values are means ± one standard deviation.

gests that intracellular citrate in the kidney is increased in metabolic alkalosis as has been suggested previously(3). The other organs tested showed either a small increase or no increase in tissue citrate after sodium bicarbonate administration.



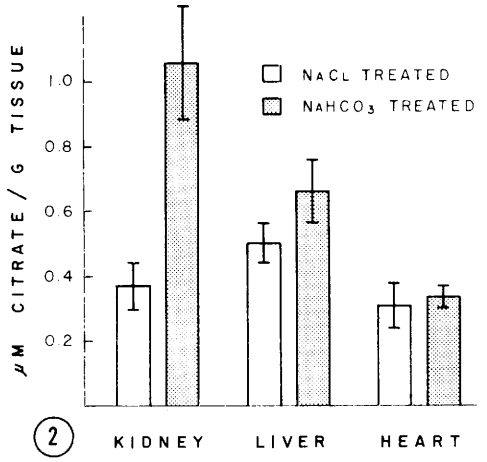


FIG. 1. Recovery of respiratory $C^{14}O_2$ after intravenous administration of citrate-1,5-C^{14}. Vertical lines represent one standard deviation from the mean.

FIG. 2. Citrate concentrations in kidney, liver and heart after administration of NaCl or $NaHCO_3$. Vertical lines represent one standard deviation from the mean.

A marked decrease in renal utilization of citrate after bicarbonate administration was demonstrated both by the increase in urinary citrate and by the increase in labelled organic acid excretion. The increase in radioactivity in the urine accounted for most of the decrease in recovery of respiratory $C^{14}O_2$. This suggests that, apart from the kidney, there was little decrease in the oxidation of radioactive citrate after bicarbonate administration. The effect of bicarbonate on tissue citrate levels also is compatible with the hypothesis that bicarbonate exerts considerable effect on citrate metabolism in the kidney but only a small effect elsewhere in the body.

Summary. Sodium bicarbonate administration increased the excretion of C^{14}-labeled citrate in the urine. It decreased the conversion of C^{14}-labeled citrate to $C^{14}O_2$ to approximately the same extent as it increased the urinary excretion of radioactive citrate. Tissue levels of citrate in the kidney rose 3-fold after sodium bicarbonate administration; there was a significant but relatively small rise in liver citrate and no change in heart citrate after sodium bicarbonate. Thus sodium bicarbonate appears to affect citrate metabolism predominantly in the kidney.

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