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Metabolism of Free Citric Acid in the Rat.*

CARL A. KUETHER, CURTIS E. MEYER AND ARTHUR H. SMITH.
From the Department of Physiological Chemistry, Wayne University College of Medicine, Detroit.

Studies in which pure citric acid was given by mouth to human subjects have shown that citric acid may be almost completely metabolized.[1-5] In the dog, large amounts of orally administered citric acid are likewise destroyed, less than 1% of the compound given appearing in the urine and no "extra" citric acid in the feces.[6] Similar results have been obtained with rabbits[7] and swine.[8, 9] The possibility that the citric acid administered was not absorbed was precluded by Langecker[7] who found a prompt and prolonged rise in the level of blood citrate and showed further that the enzymes and bacteria present in the intestinal tract of the rabbit do not destroy citric acid even when incubated for as long as 9 hours. The following experiments were conducted in order to determine whether or not the rat also possesses the ability to metabolize free citric acid.

A series of rats maintained in metabolism cages was fed the following diet *ad libitum:* lactalbumin (Borden No. 15-42) 15.0, dextrin 50.5, hydrogenated fat (Crisco) 27.0, cod liver oil (Mead) 5.0, and salt mixture[10] 2.5%. This diet was supplemented daily with 2 cc of a solution containing 80 mg of liver extract (Lilly 343) and 200 mg of Ryzamin-B (Burroughs Wellcome).

The cages were placed over large glass funnels containing a 3/16

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7 Langecker, H., *Biochem. Z.*, 1934, **273**, 43.

8 Fürth, O. von, Minnibeck, H., and Edel, A., *Ibid.*, 1934, **269**, 379.

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10 Hubbel, R. B., Mendel, L. B., and Wakeman, A. J., *J. Nutrition*, 1937, **14**, 273.

inch mesh screen which separated the feces from the urine, which was collected in a flask containing about 20 cc of 5% sulfuric acid. The funnels were rinsed down daily with distilled water. Citric acid was determined by the method of Pucher, Sherman and Vickery,[11] the final measurement being made in a photelometer using a filter with maximum transmission at 4250 Å.

After an adjustment period of 4 days, urine samples were collected for four 2-day intervals followed by 3 similar periods during which the rats received by stomach tube 400 mg per day of citric acid dissolved in distilled water to make 2 cc. Three more control samples were then collected. The urine samples were diluted to 200 cc and 2 cc aliquots taken for analysis. Later another group of animals was treated by the same procedure except that citric acid determinations were run on both the urine and the feces.

In order to show that the citric acid administered was actually absorbed and not destroyed by the bacteria in the gut, the intestinal contents of two rats were removed. One lot was mixed with citric acid and divided into 4 samples, 2 of which were analyzed immediately for citric acid and the other 2 incubated at 37° for 44 hours in glass stoppered flasks to prevent evaporation. The pH of the other sample of intestinal contents was measured, citric acid added and the pH brought back to the same point by addition of NaOH. This second sample was then treated the same as the first, divided into 4 parts, 2 analyzed immediately and 2 incubated at 37° for 44 hours. The citric acid found in the unbuffered control and incubated samples was 64.7 and 62.7 mg per g respectively and in the buffered samples 62.4 and 62.5 mg per g, showing that the intestinal contents of the rat do not destroy citric acid either at the normal pH of 6.5 or at the pH of 3.3 obtaining after the addition of citric acid.

Urinary pH was measured with a glass electrode on several animals before and after citric acid feeding, the urines being collected under toluene in large test tubes. Only samples uncontaminated with food or feces were used. The values differed by no more than 0.1 pH.

In Table I, the first line for each rat is the average intake and excretion of citric acid for three 2-day control periods immediately preceding the experimental periods. During these control periods each rat was excreting more citric acid in the urine than it was absorbing from the gut and consequently must have been synthesizing citric acid (Smith and Meyer[12]). The following 3 lines for

[11] Pucher, G. W., Sherman, C. C., and Vickery, H. B., *J. Biol. Chem.*, 1936, **113**, 235.

[12] Smith, A. H., and Meyer, C. E., *J. Biol. Chem.*, 1939, **131**, 45.

TABLE I.
Disposition of Citric Acid by the Rat.

Rat	Wt	Citric acid intake Mg per 2 days			Citric acid excretion Mg per 2 days			C.A.	Excretion of citric acid in % of total intake		
		Food	Extra	Total	Urine	Feces	Total	Abs.%	Urine	Feces	Total
8	256	17.7	0	17.7	25.1	0.84	25.9	95.5	148	4.97	153
		16.3	800	816	20.6	1.03	21.6	99.9	2.46	.123	2.58
		12.9	600	613	31.8	0.56	32.4	99.8	5.19	.091	5.28
		12.2	800	812	39.0	0.62	39.6	99.8	4.81	.076	4.88
9	232	17.0	0	17.0	22.3	1.28	23.6	92.4	142	8.15	150
		15.0	800	815	30.3	1.13	31.4	99.9	3.72	.139	3.86
		12.2	800	812	32.8	0.95	33.8	99.9	4.05	.117	4.17
		12.2	800	812	38.0	0.52	38.5	99.9	4.68	.064	4.74
10	196	17.0	0	17.0	28.7	1.31	30.0	92.4	183	8.34	191
		13.6	600	614	19.1	1.20	20.3	99.8	3.11	.196	3.30
		5.5	800	805	12.2	0.63	12.8	99.8	1.52	.078	1.60
		5.5	800	805	13.6	0.56	14.2	99.9	1.69	.070	1.76
11	156	10.2	0	10.2	19.3	0.43	19.7	96.1	197	4.39	201
		4.8	800	805	15.7	3.49	19.2	99.6	1.96	.435	2.39
		5.5	800	805	18.2	12.35	30.6	98.5	2.29	1.56	3.85
		6.8	400	407	24.2	0.23	24.4	99.9	5.94	.056	6.00
12	290	20.4	0	20.4	74.7	1.72	76.4	91.6	399	9.20	408
		17.7	400	418	60.1	1.10	61.2	99.7	14.4	.264	14.7
		12.9	800	813	52.3	0.97	53.3	99.9	6.44	.119	6.56
		12.9	800	813	72.1	1.72	73.8	99.8	8.86	.212	9.08

each rat give the figures for individual periods during which the rats were receiving extra citric acid. During these periods, with one exception (Rat 11, 3rd period, when he had diarrhea) the absorption of the administered citric acid exceeded 99%, and the percentage of the absorbed citric acid which was excreted in the urine dropped to a very low value. In all 4 of the animals in which only urinary citric acid was followed, there was actually a decrease in the absolute amount of citric acid excreted following the feeding of free acid, and rats 10 and 12 in the table show the same result. In all of the animals, feeding of extra citric acid brought about a remarkable decrease in the percentage of the absorbed citric acid appearing in the urine, showing that under the experimental conditions imposed, the rat possesses the ability to metabolize free citric acid almost completely.

Summary. Citric acid administered to the rat is absorbed since no extra citric acid appears in the feces and intestinal contents do not destroy it. The albino rat has the ability to completely metabolize maximum non-fatal quantities of free citric acid.