

## 9305 P

## On the Site of the Formation of Citric Acid in the Animal Body.

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Recent studies<sup>1, 2</sup> have demonstrated that the intravenous injection into dogs of the sodium salts of certain dicarboxylic acids, including malic acid, produces a marked and prompt increase in the amount of citric acid excreted in the urine. Simultaneous determinations of the citric acid content of urine and blood<sup>2</sup> suggested that the kidney played an important part in the formation or elimination of citric acid after the injection of sodium malate. The following experiments were designed to more definitely elucidate the question of the site of citric acid formation under these experimental conditions.

Adult male rats weighing from 250 to 450 gm. were given a citrate-low synthetic ration (casein, 18%; dextrin, 51%; Crisco, 27%; Wesson salt mixture, 4%) for a 5-day preliminary period. The animals were then lightly anesthetized with ether and sterile solutions of either sodium chloride (2.5%) or sodium malate (5%) were injected into the heart in amounts containing 100 mg. of sodium per kilo body weight. Preliminary studies showed that, under these conditions, the rat, like the dog, excretes relatively large amounts of citric acid in the urine after the parenteral administration of sodium malate. In the present experiments, the animals were again anesthetized 15 minutes after the injection of the chloride or malate, exsanguinated by bleeding from the abdominal aorta, and the tissues to be analyzed were removed and quickly frozen with carbon dioxide ice. The tissues were then pulverized, extracted with dilute sulfuric acid, and the citric acid content of aliquots of the protein-free (trichloroacetic acid) filtrates was determined. The amount of citric acid in trichloroacetic acid filtrates of the oxalated blood was also determined.

Table I shows that there was little difference between the citric acid content of the liver, muscle, and blood of the chloride-injected control rats and that of the malate-injected animals, whereas the citric acid content of the kidneys of the latter group was approximately twice that of the controls.

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<sup>1</sup> Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1937, **117**, 555.

<sup>2</sup> Orten, J. M., and Smith, A. H., Presented before Am. Soc. Biol. Chemists, Memphis, Tenn., April 23, 1937. Proceedings, p. lxxiv.

TABLE I.  
Citric Acid Content of Tissues.\*  
(Expressed as mg. %.)

Tissue	Intact Rats				Nephrectomized Rats			
	Sodium Chloride Injected		Sodium Malate Injected		Sodium Chloride Injected		Sodium Malate Injected	
	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range
Liver	1.6	1.0-2.2	1.4	1.2-1.7	2.5	1.7-3.1	2.3	1.4-2.7
Kidney	5.5	3.0-7.0	11.7	8.0-16.1	—	—	—	—
Muscle	2.7	1.7-3.7	2.6	1.5-2.9	4.2	2.9-5.2	3.3	2.2-4.2
Blood	4.0	3.3-4.8	4.0	3.4-4.3	4.6	2.9-7.5	4.7	2.5-6.3

\*Averages represent 5 rats in each group except those for kidneys of intact rats, where 8 animals were used.

In order to determine whether the "extra" citric acid present in the kidney after malate injection may have been formed elsewhere and was merely being cleared from the blood by the kidneys, further studies were made on a second series of rats treated in the same manner as those just described, but which were bilaterally nephrectomized immediately preceding the injection. The table shows that again there was little difference between the citric acid content of the blood, liver, and muscle of the control and malate-injected animals.

Thus it is evident that under the conditions employed, the citric acid formed following the injection of sodium malate into rats is produced chiefly, if not entirely, in the kidney.

9306

### On Factors Limiting Bacterial Growth. I.

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There is as yet no agreement among bacteriologists as to the specific physical and chemical influences responsible for changes observed in the rate of growth during the life of the bacterial culture.<sup>1</sup> In Fig. 1 typical curves are presented, illustrating these changes as they occurred during the development of a broth culture of *Bacterium coli*. The plotted values were calculated by us from the data of Martin,<sup>2</sup> by means of the formula  $B-A/T$  for the rate of population-increase,

<sup>1</sup> Topley and Wilson, *The Principles of Bacteriology and Immunity*, 1929, **1**, 75.

<sup>2</sup> Martin, D., *J. Gen. Physiol.*, 1931, **15**, 697.