gonadotropic serum injected and the gonads remain in an atrophied condition. When the antigonadotropic serum is discontinued the increased gonadotropic hormone being produced by the pituitary gland is not neutralized and as a result the gonads are stimulated to develop from an atrophied condition to one much greater than that found in normal animals of the same age. Further experiments are being made to determine whether the stimulation of the gonads resulting after the treatment with antigonadotropic serum is temporary or permanent.

Summary. Pretreatment of young female rats with antigonadotropic serum induced a hypersecretion of the gonadotropic hormone of the animal's pituitary. This effect was determined by the precocious development of the ovaries of the treated rats after discontinuing the injections, and by the ovarian hypertrophy occurring in female rats paired to pretreated male or female littermates. A theory is offered to explain the results obtained.

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On the Site of Formation of Citric Acid in the Dog.

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With the advent of methods of analysis suited to the determination of small amounts of citric acid,[1-3] interest in this compound and its salts as factors in intermediary metabolism has increased. The wide distribution of citric acid in the animal body has been indicated by Smith and Orten.[4] In addition, these authors[5] have shown that, in the dog following the rapid intravenous injection of the sodium salts

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1 Thunberg, T., *Biochem. Z.*, 1929, **206**, 109.

2 Kuyper, A. C., and Mattill, H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 863.

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

4 Smith, A. H., and Orten, J. M., *J. Nutr.*, 1937, **13**, 601.

5 Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1937, **117**, 555.

of a series of organic acids, the output of citric acid in the urine is increased. This synthesis was shown to be particularly striking with malate, malonate, succinate, fumarate and maleate. When sodium malate was injected the maximum rate of excretion of citric acid occurred within 60 minutes after injection and returned to normal after 4½ to 6½ hours.[6]

Krebs, Salvin and Johnson[7] showed that l-malic acid was changed to citric acid in the rat and recently Orten and Smith[8] demonstrated that the rat like the dog possesses the ability to form citric from dl-sodium malate. By means of comparative tissue analysis after intracardiac injection of sodium chloride into one group of animals and sodium malate into a second group, it was shown that renal tissue contained definitely more citric acid than did blood, muscle or liver. Similar analyses after bilateral nephrectomy led to the conclusion that "under the experimental conditions employed, the citric acid formed following the injection of sodium malate into rats either is produced chiefly in the kidney or is dependent upon the presence of the kidney for its formation."

A similar demonstration of the locus of formation of urinary citric acid from injected sodium malate has not been made in the dog. In view of the extreme rapidity with which the change has been shown to occur in this species, it was thought that, if the kidney is the site of this transformation, simultaneous analyses of the blood in the renal vein and in the femoral artery during the stage of most rapid formation of urinary citric acid, might reveal a difference in concentration of this substance attributable to "reverse leakage," so to speak, much as was found by Nash and Benedict for ammonia.[9]

In the present study 4 female dogs were used; in each animal one kidney was exteriorized in order to facilitate securing the samples of renal venous blood. Dogs A and S were prepared according to the method described by Rhoads[10] with the other kidney intact and in the normal site.‡ Dogs B and D were prepared in a generally similar way except that part of the 10th, 11th, and 12th ribs on the side of the transplanted kidney were removed to relieve tension. Also in Dogs B and D, the right kidney was removed after the other

6 Smith, A. H., and Orten, J. M., *J. Biol. Chem.*, 1938, **124**, 43.

7 Krebs, H. A., Salvin, E., and Johnson, W. A., *Biochem. J.*, 1938, **32**, 113.

8 Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1939, **128**, 101.

9 Nash, T. P., Jr., and Benedict, S. R., *J. Biol. Chem.*, 1921, **48**, 463.

10 Rhoads, C. P., *Am. J. Physiol.*, 1934, **109**, 324.

‡ We are indebted to Dr. J. C. Hinsey of the Cornell University Medical School for the use of Dogs A and S. The experiments on them were carried out in the Laboratory of Physiological Chemistry, Yale University School of Medicine.

had been exteriorized, thereby restricting the synthesis of citric acid to the kidney, the blood of which could be analyzed. Ample time was allowed for the remaining kidney to assume the entire renal excretory function of the body. All of the dogs were given the citrate-low ration used previously by Orten and Smith[5] in such quantity that body weight was maintained.

Citric acid was estimated directly on suitable aliquots of catheter specimens of urine and on trichloracetic acid filtrates of blood. The method of Pucher, Sherman and Vickery[3] was used, the final measurements being made in the early experiments with a Pulfrich spectrophotometer and in the later studies with a photoelectric colorimeter using a color filter with maximum absorption at 4250Å.

The plan of the experiments involved the simultaneous determination of citric acid in arterial and renal venous blood of unanesthetized dogs at the height of citric acid synthesis following the intravenous injection of dl-sodium malate. Five per cent dl-sodium malate in amounts providing 100 mg sodium per kg body weight was injected.

The blood samples were taken one-half hour after the injection in Dogs A and S and one hour after injection in Dogs B and D. All urine samples represented the one hour collection immediately following the injection. In Dogs A and S prior control experiments were carried out with sodium chloride in which 100 mg sodium per kg body weight in 2.5% solution was injected.

The results are summarized in Table I. It is clear that under the experimental conditions employed, there is little if any significant difference between the citric acid concentration in arterial blood and that in renal venous blood at the time when large amounts of this acid are being excreted in the urine. The data do not show that the kidney is contributing citric acid to the systemic circulation either under basal conditions (after the injection of sodium chloride) or when there is a rapid transformation of malic acid to citric acid.

Although the results of the present study do not show that citric

TABLE I.

Dog	Treatment	Blood citric acid (mg%)		Urine citric acid (mg/hr)
		Fem. Art.	Renal vein	
A	NaCl	3.0	2.7	1.4
S	"	2.5	2.2	1.1
A	No malate	4.4	3.1	226.0
S	"	2.5	2.0	156.0
B*	"	1.4	1.4	98.0
D*	"	1.5	1.3	75.0

* Unilateral nephrectomy after explantation of other kidney.

acid is formed in the kidney under the conditions of the experiment, they do not constitute evidence to the contrary. It remains for studies based upon a different experimental approach to demonstrate that in the dog, the kidney is concerned in the formation of citric acid from sodium malate administered intravenously. If the dog should prove to resemble the rat in this respect, the present study would indicate that the kidney is highly efficient, inasmuch as it removes the citric acid it has formed without returning a detectable amount to the systemic blood, even when one kidney is required to carry the entire burden (Dogs B and D) of synthesis and excretion.

The data might be interpreted as suggesting that citric acid may be formed elsewhere in the body. This point is supported by the work of Martensson[11] who worked with anesthetized fasting cats and found increases in citric acid in arterial blood over that in renal venous blood of magnitudes which may be significant. Furthermore, the gradual increase in the citric acid content of liver, blood and muscle after bilateral nephrectomy and injection of sodium chloride and sodium malate[8] also supports this point. The fact that Orten and Smith[8] state that "there *may* be an extra-renal source of citric acid" appears to have escaped the attention of Martius[12] who attributes to them the statement that citric acid originates only in the kidneys.

Summary. A comparison has been made of the citric acid content of renal venous blood with arterial (femoral) blood in dogs with an exteriorized kidney after the injection of dl-sodium malate. The venous blood was found to contain no more than or, perhaps, somewhat less citrate than arterial blood.

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Effect of Spinal Anesthesia on Intestinal Activity.

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Clinicians have long had the impression that spinal anesthesia causes increased intestinal contractions by release of the sympathetic inhibition of the intestines. This conception with the favorable re-

11 Martensson, J., *Skand. Arch. Physiol.*, 1938, **88**, 303.

12 Martius, C., *Angew. Chemie*, 1939, **52**, 223.