

A New Method for the Determination of Allantoin in Dog's Urine.**FRANK W. ALLEN AND LEOPOLD R. CERECEDO.**

(Introduced by Carl L. A. Schmidt.)

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Fosse and Bossuyt¹ have described a new method for the determination of allantoin. In their procedure allantoin is first hydrolyzed by alkali to allantoic acid. In a second step the allantoic acid is broken down by acid hydrolysis to urea and glyoxylic acid. By estimating colorimetrically the amount of glyoxylic acid present, Fosse and Bossuyt calculate the quantity of allantoin originally present in the sample analyzed.

The technique of Fosse and Bossuyt in our hands did not yield satisfactory results. Besides, we preferred to determine urea rather than glyoxylic acid as an index of the amount of allantoin present in urine.

The new procedure is as follows: To 10 cc. of diluted dog's urine 5 cc. of a 10% solution of urease (Squibb) are added. The mixture is incubated at 38° for 1½ hours. The protein is then precipitated with Tanret's Reagent and filtered off. An aliquot part of the filtrate is neutralized with N KOH using phenolphthalein as indicator, and an excess of alkali added so as to obtain a final concentration of 0.2 N KOH. The mixture is kept for 2 hours at 70°. It is then neutralized with N HCl, and an excess of acid added until the acidity of the solution is 0.1 N. Warming at 70° for 30 minutes hydrolyzes the allantoic acid present to urea. From the amount of urea present, estimated according to the method of Allen and Luck², the quantity of allantoin originally present in the sample can be calculated.

By this procedure we are able to estimate added amounts of allantoin in dog's urine in yields better than 97%. The quantity of allantoin excreted by dogs, weighing from 8 to 20 kg. in 24 hours ranges between 200-1400 mg., depending upon the total nitrogen per diem.

¹ Fosse, R., and Bossuyt, *Compt. rend. Acad.*, 1929, **188**, 107.

² Allen, F. W., and Luck, J. M., *J. Biol. Chem.*, 1929, **82**, 693.