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Determination of Allantoin in Urine.

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In the course of studies involving allantoin, difficulty was experienced in finding a suitable method for the determination of this substance in urine. The method recently introduced by Larson,[1] while including such favorable features as rapidity and the avoidance of the hydrolysis of allantoin, was not found satisfactory under varying experimental conditions. Since the Folin ammoniacal copper reagent employed in this method is not specific for allantoin, further removal of interfering substances from the urine prior to its addition to the copper reagent seemed desirable. A method involving this modification is the basis of the present report.

Following the precipitation of interfering substances with phosphotungstic acid and lead acetate, the removal of excess of the heavy metals is effected with hydrogen sulphide. The allantoin is then precipitated with mercuric acetate, the precipitate washed and redissolved with hydrochloric acid. The solution is then freed of mercury by means of hydrogen sulphide, and the allantoin in a relatively pure solution is determined colorimetrically with the Folin ammoniacal reagent adopted for this purpose by Larson.

The amount of phosphotungstic acid to be added to the urine is determined by preliminary tests as follows: To a series of 6 tubes add 2 cc. of neutral urine. To the first add one cc. of 10% phosphotungstic acid and to the others increasing amounts, namely 1.5, 2.0, 2.5, 3.0, and 3.5 cc. The tubes are shaken, allowed to stand for 5 minutes, and then filtered. To each of the clear filtrates, a few drops of 10% phosphotungstic acid are added. The least amount of phosphotungstic acid that yields a filtrate remaining clear despite the further addition of the acid determines the amount necessary for precipitation of interfering substances from the urine sample. A large excess of phosphotungstic acid must be avoided since it leads to bulky precipitates, which cause a loss of solution volume. If no one of the filtrates remains clear after the addition of the acid, the urine is too concentrated and must be diluted.

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1 Larson, H. W., *J. Biol. Chem.*, 1932, **94**, 727.

Ten cc. of neutral diluted urine are transferred to a 15 cc. centrifuge tube and dry phosphotungstic acid is added in an amount determined by the above procedure. The mixture is thoroughly stirred and allowed to stand for about 15 minutes. It is then centrifuged for 10 minutes. The supernatant fluid is transferred to a 15 cc. graduated centrifuge tube and the volume noted (V_1). The supernatant fluid in every determination is tested with a few crystals of phosphotungstic acid to ensure that all interfering substances precipitable by this reagent have been removed.

One cc. of 50% basic lead acetate is now added to the supernatant fluid in the graduated centrifuge tube. The mixture is thoroughly stirred and tested with litmus paper. If not alkaline, it is made so by the further addition of lead acetate, drop by drop. The volume of lead acetate that has been added is noted (V_L) and the mixture centrifuged. The clear supernatant fluid is transferred to another 15-cc. centrifuge tube and treated with hydrogen sulphide until all the heavy metals have been removed. The precipitate is allowed to settle slightly and with the aid of a glass rod the sulphide clinging to the sides of the tube above the level of the liquid is brushed down. The mixture is centrifuged for about one minute and the supernatant fluid transferred to another 15-cc. graduated centrifuge tube. The solution is aerated for about 15 minutes and the volume noted (V_2). One drop of phenolphthalein is added and the solution titrated with 2 N NaOH until a permanent pink is obtained. Five cc. of a mercury reagent containing 100 gm. mercuric acetate and 300 gm. sodium acetate per liter of solution are now added. The mixture is stirred and allowed to stand for 10 minutes. Following centrifugation, the clear supernatant fluid is discarded. The precipitate is then stirred with alkaline water and the mixture centrifuged. If the urine contains glucose, the precipitate is washed again. The wash water is prepared by adding 5 drops of 2 N NaOH to one liter of water.

Four cc. of water are added to the precipitate, followed by 2 drops of concentrated HCl. The contents of the tube are stirred until the precipitate is dissolved. A faint opalescence may remain at this point. Water is now added to bring the volume to 5.0 cc. (V_3). If allantoin is present in a concentration greater than 0.5 gm. per liter, the volume at this point is made up to 10 cc. The mercury is precipitated with hydrogen sulphide and the tube centrifuged. The clear supernatant fluid is decanted into another tube and aerated for 15 minutes. A 2-cc. sample is then treated with the copper reagent according to Larson's method, and the allantoin content compared colorimetrically with a standard containing 1.0 mg. allantoin.

The allantoin in milligrams in n cc. of diluted urine may be calculated as follows:

$$n \times \frac{S}{U} \times \frac{V_1 + V_L}{V_1} \times \frac{V_3}{V_2} \times \frac{1}{2},$$

where S and U are the readings of the standard and unknown respectively.

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Effect of Insulin on Excretion of Allantoin by the Normal Dog.

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The influence of insulin on purine metabolism has been the subject of few investigations. Although Kurti and Gyorgyi[1] found no constant effect of insulin on the endogenous excretion of uric acid in man, they reported that insulin led to an increase in blood uric acid during the ingestion of nucleic acid. Quick[2] has shown that 30 units of insulin along with a high carbohydrate diet had no immediate effect on the uric acid output by man. In the dog, Taubmann[3] reported that insulin led to an increased excretion of allantoin, whereas Ogawa[4] found a decrease following the administration of this hormone.

In the present investigation the dogs were fed a Cowgill diet and insulin was injected after nitrogen and allantoin equilibrium had

TABLE I.

Excretion of Allantoin Nitrogen (Dog. L).

Date	1:35 p.m. to 8:35 a.m.	8:35 a.m. to 1:35 p.m.	Total
Jan. 8	.118	.029	.147
" 9	.112	.026	.138
" 10	.117	.028	.145
" 11*	.119	.062	.181
" 12	.124	.025	.149
" 13	.118	.028	.146

* Insulin injected.

1 Kurti, L., and Gyorgyi, G., *Klin. Wochenschr.*, 1927, **6**, 1426.

2 Quick, A. J., *J. Biol. Chem.*, 1932, **98**, 157.

3 Taubmann, G., *Arch. f. exp. Pathol. u. Pharmak.*, 1928, **132**, 124.

4 Ogawa, T., *Folia Endocrinol. Japon.*, 1931, **7**, 7.