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A procedure for the detection of allantoin in body fluids.

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None of the ordinary constituents of body fluids interferes with the test outlined below, save a substance in the urine, as yet unidentified, but which is probably a small amount of oxalic and perhaps glyoxalic acids, which have been reported as occuring freely in urine by Fürbinger[1] and by Granström[2], respectively. Creatinin, creatin, uric acid, etc. are negative. Allantoin, however, may be separated from the other constituents of the urine by precipitation with ammonical silver nitrate. Then the allantoin is subjected to the test. This procedure is necessary only in the case of urine for blood and other body-fluids show no interfering substance.

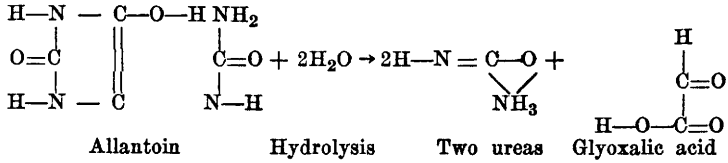
To 25 mg. filtered urine, add, if necessary, 10 per cent hydrochloric acid to slight acidity; if the urine is initially acid, this is unnecessary. Then add, drop by drop, 1 per cent aqueous solution silver nitrate until no more precipitate of silver urate is obtained; ten drops are usually sufficient. Let stand five minutes, filter, saving the filtrate. Or, one may use a 50 mg. centrifuge tube and precipitate by means of the centrifuge. A test for the presence of uric acid on this filtrate may be made by Folin's test. Then add, drop by drop, concentrated ammonium hydroxid to the filtrate to obtain the precipitate of allantoin silver nitrate; in case, after the addition of two drops of the ammonia, no precipitate appears, add a drop or two of silver nitrate solution as before, to insure that all of the silver nitrate first used was not carried down as silver urate. An excess of ammonia causes solution of the precipitate.

A precipitate, varying with the amounts of allantoin, may be obtained by centrifuging. Procedure for all fluids: To a few mls. of the fluid, or to the precipitate obtained above after decantation of the supernatant fluid, add one small flake of indol,

[1] Fürbinger, P., and Dunlop, J., *Deutsch. Arch. f. Klein. Med.*, 1876, xviii, 143-192. Dunlop, J., *J. Path. and Bact.*, 1895-6, iii, 389,429.

[2] Granström, E., *Hofmeister's Beitrage*, 1907-8, xi, 137-142.

a commercial product. To the fluid add, drop by drop carefully down the sides of the tube and inclining it, concentrated sulphuric acid, permitting the liquids to layer. If a precipitate of allantoin silver nitrate is used, dilute with about 2 mg. of distilled water before adding the sulphuric acid. At the point of junction of the acid and the supernatant fluid, a colored zone appears if the test is positive for allantoin, due to the hydrolysis of this substance in the warm acid to glyoxalic acid and urea and the condensing of the glyoxalic acid with indol in a manner similar to that in the Hopkins-Cole reaction for proteins, in which tryptophan affords the indol ring; the reaction is as follows (After Grimaux[3]):



If desirable, a 1:1000 per cent solution of indol in water may be used in place of the substance itself. Five drops of this solution may be used in the test. Like nearly all colorimetric procedures involving an aldehyde, this test does not lend itself to quantitative procedure.

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The lysis of dead bacteria by bacteriophage.

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There is no difference of opinion that only young and actively growing bacteria are subject to transmissible lysis. Old or dead bacteria do not undergo lysis and do not contribute to the increase in concentration of the active lytic substance, but rather, on the contrary, bring about a measurable reduction of it.

[3] Grimaux, E., *Ann. J. Chim.*, 1877, xi, 389.