Uric Acid

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Principle

The enzyme uricase catalyses the reaction:

(1) $$\text{Uric acid} + O_2 + 2\,H_2O \longrightarrow \text{allantoin} + H_2O_2 + CO_2$$

The measure of the reaction is the decrease in optical density at 293 mμ due to the oxidation of uric acid. This decrease is proportional to the uric acid concentration.

Reagents *)

1. Glycine, A. R.
2. Sodium hydroxide, A. R., 1 N
3. Uricase
   lyophilized preparation in ampoules or as a suspension in 10% ammonium sulphate solution. Commercial preparation, see p. 1000.

Preparation of Solutions

I. Glycine buffer (0.07 M; pH 9.3):
Dissolve 25 g. glycine in 200 ml. CO_2-free distilled water and add 110 ml. 1 N NaOH.

II. Uricase (200–300 units **)/ml.):
Dissolve the contents of a uricase ampoule (75 units) with 0.25 ml. buffer (solution I). Or use the enzyme suspension undiluted.

Stability of the solutions

The enzyme solution is stable for at least 14 days in a refrigerator. The buffer keeps for several months providing bacterial contamination is avoided.

Procedure

Experimental material

Dilute plasma or serum (to contain 0.5–5.0 μg. uric acid/ml.) 1:40 with buffer (solution I). Dilute urine (to contain 2–4 μg. uric acid/ml.) 1:400 with buffer (solution I). If the urine contains a large sediment and therefore a large amount of uric acid, dilute 1:10^3. Dilute urine which is practically colourless 1:100. Further treatment is unnecessary.

Spectrophotometric measurements

Wavelength: 293 mμ; silica or glass cuvettes, light path: 1 cm.; final volume: 3.01 ml.; room temperature. Measure against a control cuvette.

The enzyme absorbs at 293 mμ. Although the optical density of the enzyme E_E is usually negligible, it should be determined for each enzyme preparation.

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*) Complete reagent kits are commercially available (see p. 1036).

**) A unit is the amount of enzyme which oxidizes 1 μg. uric acid/min. at pH 9.3 (initial amount of uric acid = 5 μg.). At 293 mμ a uricase unit causes an optical density change of 0.025 /min. in a 3 ml. assay mixture with a 1 cm. light path.

Pipette into the cuvette:

2.99 ml. buffer (solution I)
0.01 ml. uricase solution or suspension *) (II) (2—3 units).

Read the optical density E_E against a blank cuvette containing 3 ml. buffer (solution I).

As the samples normally have a very high optical density at 293 mμ (due to protein and other absorbing constituents) measurements are made against a control cuvette containing less sample than the experimental cuvette.

Pipette successively into the cuvettes:

Control cuvette: 2.40 ml. dilute serum or plasma
0.60 ml. buffer (solution I)
or 3.00 ml. distilled water for measurements of uric acid in urine

Experimental cuvette: 3.00 ml. dilute sample.

Read the optical density E_1 against the control cuvette **). Mix into the experimental cuvette

0.01 ml. uricase solution or suspension (II) (2—3 units).

On completion of the reaction (10—20 min.) read the optical density E_2. $E_1 + E_E - E_2 = \Delta E$ is used for the calculations.

If with large amounts of uric acid in the sample the value for E_2 is negative, read the optical density of the control cuvette against the experimental cuvette. ΔE is then $E_1 + E_E + E_2$. If the reading is off the scale, then measure the optical density E_3 of the experimental cuvette against a water blank and determine the optical density difference ΔE_C of the control cuvette against the water blank. Then $\Delta E = E_1 + E_E - E_3 + \Delta E_C$.

Calculations

With a 1 cm. light path the oxidation of 1 μg. uric acid/ml. corresponds to an optical density decrease of $\Delta E = 0.075$ at 293 mμ.

Therefore $$\frac{\Delta E}{0.075} = \mu g.\ \text{uric acid/ml. assay mixture.}$$

Owing to the preliminary dilution of the samples (plasma and serum 1:40, urine 1:400) it follows that:

$$\frac{\Delta E}{0.075} \times 40 = \mu g.\ \text{uric acid/ml. serum or plasma}$$

$$\frac{\Delta E}{0.075} \times 400 = \mu g.\ \text{uric acid/ml. urine.}$$

Multiplication of these values by 0.1 gives the results in the usual clinical units of mg.%.

Sources of Error

The determination is not interfered with by any substances present in the samples.

Specificity

Uricase is specific for uric acid.

*) The optical density of the amount of enzyme used for the assay must be small in relation to the measured optical density changes.

**) Usually values between 0.500 and 0.100 are obtained. If the optical densities are higher or lower vary the volume of sample in the control cuvette accordingly.