Specific determination of plasma and urinary lactose

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The recognition of three cases of lactose intolerance in the Sheffield Children’s Hospital led to an investigation into the specific determination of plasma and urinary lactose levels following lactose loading tests.

Using the method of Reithel (1962) (see below) it was found that aqueous solutions of lactose (0.1 ml.) were readily and consistently determined even at levels down to 5 mg. per 100 ml.

As plasma glucose interferes with the determination it was destroyed by glucose oxidase and the oxidase itself was removed during the subsequent deproteinization.

DETERMINATION

The method depends upon the measurement of glucose liberated from lactose by hydrolysis with β-galactosidase, by the following steps:—

1. Lactose→glucose + galactose. With a comparatively high concentration of hexokinase and adenosine triphosphate, the glucose is immediately converted to glucose-6-phosphate, as in step 2.

2. Glucose + A.T.P.→glucose-6-phosphate + A.D.P.

The glucose-6-phosphate produced is oxidized by glucose-6-phosphate dehydrogenase in the presence of nicotinamide adenine dinucleotide phosphate (N.A.D.P.).

3. Glucose-6-phosphate

→6-phosphogluconate + N.A.D.P.H. + H+.

The increase in the amount of N.A.D.P.H. measured by the increase in optical density at 340 μ is directly proportional to the amount of glucose liberated from the original lactose.

In Reithel’s method, reaction 1 is made rate-limiting in order to resist the transglucosidase activity of the enzyme.

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Rapid method for the estimation of plasma haemoglobin levels—concluded.

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REFERENCES


Technical methods

β-galactosidase. Plasma that has been either frozen or freshly obtained is mixed with an equal quantity of glucose oxidase reagent containing catalase. The latter reagent is included in order to give rapid decomposition of the hydrogen peroxide produced by the oxidase, because a previous series of unsatisfactory results was attributed to hydrogen peroxide or other peroxides interfering with the dehydrogenase enzyme.

The buffering capacity of the plasma is sufficient to ensure complete removal of plasma glucose by the oxidase reagent.

METHOD

REAGENTS

1 Salt solution. Potassium chloride and magnesium chloride: 20 g. MgCl₂·6H₂O and 7.5 g. KC1 per 100 ml. of deionized water.

2 Adenosine triphosphate (A.T.P.): 20 mg. per ml. in deionized water.

3 Nicotinamide adenine dinucleotide phosphate (N.A.D.P.): 20 mg. per ml. in deionized water.

4 Hexokinase: 10 mg. per ml.

5 Glucose-6-phosphate dehydrogenase: 5 mg. per ml.

6 β-Galactosidase: 20 mg. per ml. in deionized water.

7 "Tris" 2-amino-2- (hydroxymethyl) 3-propane-1,3-diol: 0.5% solution in deionized water.

8 Glucose oxidase reagent: 9 parts of Fermcozyme 5 parts catalase 1 solution.

A fresh heparinized plasma should be used immediately or stored below 0°C. The plasma (0.05 ml.) is mixed with an equal quantity of glucose oxidase reagent in a micro-tube (0.4 ml. polypropylene) and incubated at 56°C for 20 minutes, thereafter 0.05 N acetic acid (0.1 ml.) is added and the contents are mixed on a vibrator.

Deproteinization is completed by heating the tubes in a water bath at 70°C for three minutes and then cooling rapidly. After centrifugation 0.1 ml. of the supernatant is transferred to a 0.5 ml. microcell in a spectrophotometer, reading at 340 mμ (we have used the Optica instrument). An equal volume of 0.5% Tris solution is added and the contents shaken, giving a final pH of 7.4. The following solutions are then added to the cell in the stated order.

(i) Salt solution... 5 microlitres
(ii) N.A.D.P. ... 10 microlitres
(iii) A.T.P. ... 10 microlitres
(iv) Hexokinase ... 5 microlitres
(v) G-6-P.D. ... 10 microlitres

the cell volume is made up with deionised water.

The cell contents are thoroughly mixed and the absorbance is read at 340 mμ. If the reading is not steady it should be allowed to become so before the optical density is noted. At this point the absorbance at 400 mμ is also taken.

β-Galactosidase solution, 20 microlitres, is added and the absorbance at 340 mμ is allowed to become steady before reading; once it has done so a reading at 400 mμ is taken.

1 C. F. Boehringer and Sohne
2 β-galactosidase Lactase F from British Drug Houses.
3 Hughes and Hughes.
4 Sigma Chemical Co.
Technical methods

A simple device for microdialysis

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One of the problems of analysing macromolecular substances by immunodiffusion and immunoelectrophoretic techniques is that of concentrating these substances from the dilute fractions obtained off columns of ion-exchange and gel-filtration materials, or eluates of substances adsorbed to cells. The difficulty is increased when only small volumes of the starting material are available or required for analysis. Loss of material is considerable when small volumes are concentrated in cellophane bags by dialysis against polyethylene glycol. Much of it remains trapped in the folds around the occluding knots. Some of the difficulty can be avoided by the use of the simple device described.

The wide end of a conical plastic needle cover is cut off squarely (A). A 3 mm. ring is cut from this end (AB) and slid over the narrow end which is then cut off below the ring (C). The sharp outer edge of the narrow end of the tube is made smooth. A small square of dialysis membrane is applied to the narrow end of the tube and secured by means of the ring as illustrated. The sample to be concentrated is pipetted into the tube which is transferred to a 1 × 5 cm. tube containing a small quantity of wet polyethylene glycol. Within a few hours a sample volume of 0·1 ml. is concentrated tenfold. The concentrate may be removed with a fine pipette and applied directly to the well or origin of the system being used in the analysis. Alternatively, for some procedures it is convenient to leave the sample in the tube until all the water has dialysed into the polyethylene glycol. The high molecular weight material deposits as a uniform circular film on the membrane. After washing the outside of the device, to remove the polyethylene glycol, a small disc, with the deposit on it, is cut from the membrane with a sharp cork-borer. The disc may be applied, deposit side down, to an agar surface in an immunological system. In this way a large number of samples may be examined easily, and, as very little material is lost, a reasonable quantitative assessment can be made of a particular immunologically precipitating constituent.

REFERENCE

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