Suitability of the Azocoupling Reaction with 1-Naphthyl-β-D-Glucoside for the Histochemical Demonstration of Lactase (Lactase-β-Glucosidase Complex) in Human Enterobiopsies

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Summary. The suitability of the simultaneous azocoupling reaction with 1-naphthyl-β-D-glucoside and hexazonium-p-rosanilin in the detection of the activity of lactase (or lactase-β-glucosidase complex) in jejunal biopsies of patients with various forms of the malabsorption syndrome was tested. Results were compared with those obtained with the indigogenic method using 4-Cl-5-Br-3-indolyl-β-D-fucoside which is the method of choice. Both methods gave identical results as far as the relative intensity of the brush border staining was concerned. The azocoupling method applied in unfixed cold microtome sections can be recommended for the routine diagnostics of the malabsorption syndrome when the indolyl substrate is not available.

Introduction

The determination of lactase activity in enterobiopsies became a very important method in the diagnostics of the malabsorption syndrome. The activity of this enzyme can be assessed either biochemically (in homogenates, see Asp and Dahlqvist, 1974, for references) or histochemically (in unfixed cold microtome sections, see Lojda, 1974, for references).

As was shown in our previous papers (Lojda and Kraml, 1971; Lojda et al., 1973, 1974a; Lojda, 1974) the histochemical approach is very suitable in diagnostic practice. Its advantages (in comparison with the biochemical estimation of lactase activity in homogenates) are: a) It requires much less material (1 cold microtome section). b) Lysosomal acid β-galactosidase, which disturbs the correct biochemical determination (unless p-Cl-mercuribenzoate inhibition is used), does not disturb histochemical detection at all or is easily distinguished from the brush border lactase activity. c) The morphological pattern of the enterobiopsy which is indispensable for a correct interpretation (this requires a separate working out of a portion of the biopsy when the biochemical approach is used) can be assessed in one and the same section. The semi-quantitative evaluation of the activity is sufficient for practical purposes. It can be improved when the time of the appearance of the brush border staining is taken as a measure of activity or when the staining intensity is measured in a cytospectrophotometer.

For the histochemical demonstration of lactase activity an indigogenic method with 4-Cl-5-Br-3-indolyl-β-D-fucoside was elaborated (Lojda and Kraml, 1971). This method enables a correct localization of the enzyme activity in the brush
border and a more precise analysis of lactase deficiency than is possible by the biochemical approach (Lojda et al., 1974a).

Although the substrate by itself is quite expensive the histochemical demonstration of lactase activity with it turns out to be inexpensive because the medium is very stable and can be used several times (after incubation the medium is filtered and stored frozen pending further use). In fact 3 mg of the substrate proved to be sufficient for the demonstration of the lactase activity in at least 40 enterobiosies.

However, for the last two years 4-Cl-5-Br-3-indolyl-β-D-fucoside produced by Cyclo Chemicals, Division of Travenol Laboratories, Philadelphia, Pa., USA, was not supplied (nowadays it is furnished by Bachem, 4077 Glence Ave., Marina-Del-Ray, Calif., 90291, USA). Therefore another suitable histochemical method was to be searched for.

In our previous paper devoted to synthetic substrates suitable for the histochemical demonstration of disaccharidases (Lojda et al., 1973) it was shown that lactase isolated from the human intestine cleaves also glucosides (e.g. 4-Cl-5-Br-3-indolyl-β-D-glucoside, 1-naphthyl-β-D-glucoside) even when in this case the splitting rate is lower than that of fucoside. However, it is higher than the splitting rate for the respective galactosides. Although 4-Cl-5-Br-3-indolyl-β-D-glucoside is also feasible 1-naphthyl-β-D-glucoside is much cheaper. When the naphthyl substrate is applied in the simultaneous azocoupling procedure with hexazonium-p-rosanilin a very good localization of the enzyme activity in the brush border of enterocytes can be obtained (Lojda, 1972; Gossrau, 1973). A study was therefore undertaken in which results in human jejunal biopsies obtained with 4-Cl-5-Br-3-indolyl-β-D-fucoside were compared with those obtained with 1-naphthyl-β-D-glucoside.

**Materials and Methods**

195 peroral jejunal biopsies of patients with various forms of the malabsorption syndrome (both children and adults) were quenched in petrolether chilled with acetone-dry ice mixture immediately after their removal from the Crosby capsule and stretching on a gelatine foil. Biopsies were cut in a cold microtome into sections 12 μ thick which were transferred to nonprecooled slides on which they thawed immediately. Slides with sections were incubated in the following media (without any prefixation):

- **Medium with 1-naphthyl-β-D-glucoside** (Lachema, Brno, ČSSR; Koeh & Light, Colnbrook, England). A buffered solution of hexazonium-p-rosaniline [HP, consisting of 0.3 ml HP—prepared on mixing 0.15 ml of cold 4% p-rosanilin in 2N HCl and 0.15 ml of cold NaN₂—and of 10 ml 0.1 M citrate phosphate buffer pH 6.2 the pH does not need to be corrected if it is between 5.5 and 6.0; when results of several experiments are to be compared it should be adjusted precisely] was added to 4 mg of 1-naphthyl-β-D-glucoside dissolved in 0.5 ml dimethylformamide. Sections were incubated at 37°C for 2 h, at room temperature for 3 h and in a refrigerator (4–8°C) overnight. After the incubation slides were transferred to 4% formaldehyde for several hours (to minimize the formation of nitrogen bubbles). Afterwards slides were washed in water and mounted either in Apáthy's gum sirup or after dehydration and clearing in Entellan (Merck, Darmstadt, BRD).

- **Medium with 4-Cl-5-Br-3-indolyl-β-D-fucoside** (Cyclo Chemicals) was prepared according to Lojda and Kraml (1971). The solution consisting of 9 ml of 0.1 M citrate phosphate buffer pH 6.0, 0.75 ml of 1.66% potassium ferricyanide and 0.75 ml of 2.11% potassium ferrocyanide was mixed with 3 mg 4-Cl-5-Br-3-indolyl-β-D-fucoside dissolved in 0.5 ml dimethylformamide. Sections were incubated at 37°C and the time of appearance of blue