ON THE APPLICATION OF BARFOED'S REAGENT TO SHOW THE HYDROLYSIS OF DISACCHARIDES BY ENZYMES

By HERBERT E. ROAF, M.D., *Lecturer on Physiology.*

From the Physiological Department, University of Liverpool

(Received April 7th, 1908)

The methods which are used to show the hydrolytic splitting of lactose and maltose, are not so simple as those which demonstrate the hydrolysis of saccharose and polysaccharides. The reason for this is that in the latter case there is a change from a non-reducing carbohydrate to a reducing sugar, whilst in the former the original material will already reduce cupric hydrate in alkaline solution. It is, therefore, necessary to make a quantitative estimation of the reducing power in two solutions, one of which is a control containing the boiled enzyme and the other is a similar mixture containing the unboiled enzyme.¹ The presence of lactase and maltase can be just as easily proved as the ordinary amylolytic enzymes, by using copper acetate in acetic solution.² This reagent is reduced by monosaccharides, whilst under proper conditions, disaccharides leave it unchanged.

Hinkel and Sherman have recently investigated this reaction by comparing the behaviour of the reagent with glucose, maltose, lactose and saccharose.³ They found that using five cubic centimetres of the reagent the presence of 0.0004 gramme glucose can be shown, either alone, or mixed with disaccharides, provided that the total weight of disaccharide does not exceed 0.02 gramme. The period

1. Polarimetric and other tests have also been used, see Aders Plimmer, *Journ. Physiol.*, Vol. XXXV, p. 20, 1907.

2. Barfoed, *Fresenius Zeit. f. anal. Chem.*, Vol. XII, p. 27, 1873.

3. *Journ. Amer. Chem. Soc.*, Vol. XXIX, p. 1744, 1907.

of heating should, however, not be too long or reduction may occur owing to hydrolysis of the disaccharide by the acetic acid in the reagent. They also recommend that each observer should standardise his reagent before using it.

Their observations have been confirmed by me, and it seemed desirable to apply this reagent as a qualitative test,¹ when testing for the presence of lactase and maltase.

To demonstrate the presence of lactase and maltase, the following method was employed. To five cubic centimetres of a one per cent. solution of the disaccharide, one cubic centimetre of an aqueous (toluol water) extract of the first part of the small intestine was added; toluol being used as a preservative. After the period of digestion was ended, one cubic centimetre of this mixture was added to five cubic centimetres of the copper acetate reagent, and the resulting solution was placed in a boiling water bath. The tubes were examined at the end of three minutes, and if no reduction was visible they were replaced and re-examined at the end of the fourth and fifth minutes.

It was found that one cubic centimetre of a one per cent. solution of either lactose or maltose does not cause reduction until heated for nine or ten minutes. Thus, if the heating does not exceed five minutes any reduction with this strength of sugar must be due to the formation of monosaccharides. There is also a further check on the accuracy of the method, the control should show no reduction and as it is heated under identical conditions, the absence of reduction proves that the reduction caused by the other solution must be due to the hydrolytic action of an enzyme.

Experiment I.—Aqueous extract of duodenum and first portion of jejunum of cat.

Solution	At end of 1 day	At end of 3 days
5 c.c. 1 % lactose + 1 c.c. unboiled enzyme	No reduction	Slight reduction within 5 minutes
5 c.c. 1 % lactose + 1 c.c. boiled enzyme	No reduction	No reduction
5 c.c. 1 % maltose + 1 c.c. unboiled enzyme	Marked reduction within 3 minutes	—
5 c.c. 1 % maltose + 1 c.c. boiled enzyme	No reduction	—

1. This reagent might also be applied quantitatively, as glucose can be completely removed from a solution if the total amount does not exceed 0.002 g. per 5 c.c. of the reagent (Hinkel and Sherman, p. 1747) and the oxide separates in a form which could easily be filtered off and weighed.

Experiment II.—Aqueous extract of first portion of small intestine of a kitten four days old.

Solution	At end of 1 day
5 c.c. 1 % lactose + 1 c.c. unboiled enzyme	... Marked reduction within 3 minutes
5 c.c. 1 % lactose + 1 c.c. boiled enzyme	... No reduction
5 c.c. 1 % maltose + 1 c.c. unboiled enzyme	... Marked reduction within 3 minutes
5 c.c. 1 % maltose + 1 c.c. boiled enzyme	... No reduction

These two experiments show that both lactase and maltase were present in the intestine of a kitten whilst the adult cat possessed maltase, but the amount of lactase was much less than in a young animal.¹

This result, agreeing as it does with previous workers who have studied the distribution of lactase and maltase, suggests that Barfoed's reagent may be used whenever it is wished to demonstrate the hydrolysis of disaccharides.

1. Cf. Plimmer, *loc. cit.*, p. 29.