73. A Necessary Precaution in the Use of Takadiastase for the Estimation of Maltose

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Of recent years enzymic, rather than purely chemical, methods of hydrolysis have become increasingly popular in biochemical analysis, especially in that of carbohydrates and carbohydrate mixtures. It is customary to add toluene to the enzyme-substrate mixture in the hope of ensuring sterility during the period of incubation. During some investigations involving considerable numbers of incubations the writer became doubtful of the efficiency of toluene in this respect, and it was necessary in the case of the maltose-glucose hydrolysis to investigate the position more critically.

Yemm [1935] used takadiastase to hydrolyse maltose in the analysis of sugar mixtures derived from barley seedlings. He assumed that maltose, being composed of two glucose units but possessing only one reducing group, should on hydrolysis show an increase in reducing power of 100 %. His experiments, in which toluene was used as an antiseptic, gave him an increase of rather less than 100 % as measured by the Hagedorn-Jensen technique. The observed increases lay in fact between 90 and 97 % of the original value. This, he suggested, might be due to the establishment of a glucose-maltose equilibrium. This explanation involves the supposition that maltose was being synthesized to a significant extent during incubation. While such syntheses have been demonstrated, it is unlikely that in the dilute solutions now under consideration the extent of this synthesis would markedly affect the position of the equilibrium point. Yemm allowed for the discrepancy between his observed and expected results by assuming a conventional increase of 95 % and calculating accordingly. In view of the small amounts of maltose he was measuring in relation to the quantities of other sugars present, it is in the highest degree unlikely that his assumption involved any significant degree of error in his conclusions.

The present writer was induced to examine the position more closely by finding the same degree of discrepancy between the expected and observed increases in the reducing power of maltose solutions after takadiastase hydrolysis.

In the first place, the assumption that the 'theoretical' increase in reducing power on hydrolysis is 100 % is unsound. This question was investigated using the Shaffer & Somogyi [1933] method of measuring reducing power as modified by Hanes (personal communication). Reagent 60, which gives more complete oxidation of slowly reducing sugars such as maltose, was used.

Dr Hanes, in the same communication, provided a very complete set of figures for the reducing power of maltose solutions with reagent 60; these are reproduced here with his permission in Table 1. Using the same reagent, the writer has obtained figures for the reducing power of glucose (B.D.H. Analar glucose dried for 24 hr. at 80°). These figures are given in Table 2. Curves representing these sets of figures were constructed and used, by interpolation where necessary, for the calculations which follow.

The reducing power of 1 mg. maltose is equivalent to 1.75 ml. $N/100 \text{ Na}_2\text{S}_2\text{O}_3$. 1 mg. maltose will yield 1.053 mg. glucose on hydrolysis. The reducing power of 1.053 mg. glucose is 3.65 ml. $N/100 \text{ Na}_2\text{S}_2\text{O}_3$. This is an increase of 1.90 ml. or 109 %. This is therefore the 'theoretical' expectation of the increase in the reducing power of maltose after complete hydrolysis.

Table 1. *Reducing power of maltose solutions with Shaffer-Somogyi reagent 60*
(Data of C. S. Hanes)

Maltose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100	Maltose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100	Maltose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100	Maltose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100
0.1	0.17	0.5	0.85	1.4	2.50	3.0	5.56
0.2	0.34	0.6	1.03	1.8	3.26	3.5	6.55
0.3	0.51	0.8	1.385	2.2	4.03	4.0	7.57
0.4	0.68	1.0	1.75	2.6	4.80	—	—

Table 2. *Reducing power of glucose solutions with Shaffer-Somogyi reagent 60*

Glucose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100	Glucose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100	Glucose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100	Glucose mg./5 ml.	Na ₂ S ₂ O ₃ ml. N/100
0.5	1.65	1.0	3.42	1.5	5.24	2.0	7.11

The most obvious explanation of the very considerable discrepancy between 109 % and ~95 % thus revealed was bacterial contamination. The maltose and takadiastase preparations were therefore cultured on agar plates. No micro-organisms grew from the maltose, but the takadiastase preparation was found to be heavily infected. Counts of the plates indicated that there were approximately two million organisms per g. of powder.

By the use of a 'Technico' filter with Seitz E.K. bacterial filter pads and the appropriate aseptic technique, a 1 % solution of takadiastase was prepared which was shown to be sterile. It produced the maximum increase in the reducing power of maltose solutions in 42 hr. at 30° under aseptic conditions. The theoretical conclusion reached above was now examined in practice. A solution of a B.D.H. preparation of maltose had a reducing power, on appropriate dilution, of 3.25 ml. N/100 Na₂S₂O₃ per 5 ml. This is equivalent to 1.80 mg. maltose according to Hanes's figures. On incubating part of the original solution with takadiastase under sterile conditions the reducing power, again after dilution and after subtraction of the takadiastase blank value, was 6.82 ml. N/100 Na₂S₂O₃, an increase of 110 %. This corresponds to 1.92 mg. glucose; the calculated amount is 1.90 mg.

This indicates that micro-organisms were responsible for the low values found by Yemm and, initially, by the writer. It seems clear that the uncritical use of toluene in work of this kind may give rise to considerable errors which will not be constant in magnitude. It is advisable, if an aseptic technique cannot be employed, to use a more effective antiseptic such as thymol. The writer has found that this substance, if added in sufficient quantity, will ensure sterility in plant preparations and extracts, over considerable periods of incubation. The proportion of 3 parts by weight of thymol to 1000 parts of fresh material has been found, in comparative trials, to be satisfactory with preparations from seedling barley, in which biochemical reactions were being studied.

The writer is indebted to Dr C. S. Hanes for permission to quote his figures for the reducing power of maltose. His best thanks are also gratefully offered to Dr W. O. James, under whose direction and in whose laboratory the investigations were conducted.

REFERENCES

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