DILATOMETRIC STUDIES IN THE ENZYMIC HYDROLYSIS OF POLYSACCHARIDES.

Part I. Hydrolysis of Inulin.

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The hydrolysis of substrates by their specific enzymes, is accompanied by measurable changes of volume (Sreenivasaya and Sastri, 1929) which are generally found to be proportional to the quantity of substrates hydrolysed (Peter Rona and Nelly Neuenschwander-Lemmer, 1931 and Sreenivasaya and Sreerangachar, 1932). The kinetics of enzyme reactions can therefore be followed in a suitably designed dilatometer by recording the volume-changes registered by the instrument at different intervals of time. The course of the reaction can also be followed by an entirely independent physical or chemical method, usually by an estimation of one of the products of hydrolysis. The two independent sets of results thus obtained, can be correlated.

There are two classes of substrates : crystalloids like sucrose, maltose, urea, arginine and glucosides, and colloids like proteins and polysaccharides. Crystalloidal substrates which go into true solution when subjected to enzyme hydrolysis do not exhibit any abnormalities and there exists a strict linear relationship between the dilatometric changes of volume and the corresponding chemical values at all stages of the reaction.

A study of the enzymic digestion of colloids, particularly those which are hydrophillic in character, is however complicated by the fact that with the enzymic disintegration of the colloidal micellæ a disturbance of the water balance in the reacting system is created, a circumstance which influences the dilatometric measurement in the earlier stages of the reaction but does not obviously affect the values obtained by chemical analysis. The dilatometer therefore affords us a possible means of detecting these abnormal changes accompanying the hydrolysis of hydrophillic colloids. The study of tryptic hydrolysis of casein and gelatin which has been carried out by Sreenivasaya, Sastri and Sreerangachar (1934) illustrates this point.

Experimental.

The present communication deals with a dilatometric study of the enzymic hydrolysis of inulin, one of the simpler polysaccharides, by its specific enzyme inulinase. Pfanstiehl's chemically pure inulin, and inulinase extract from a fungus (*Penicillium* sp ?) were employed in the following experiments. The experimental procedure employed was the differential method fully described in one of our earlier communications (1932). The reactions were carried out at 30°C. and at the optimum pH of inulinase (pH 3·8), McIlvain's citrate buffer being employed for the purpose.

Fructose released during the enzymic digestion of inulin was estimated by Bertrand's method and as suggested by the authors (Bertrand and Thomas, 1910) the copper values for invert sugar have been adopted for calculating the quantity of fructose. Three concentrations of inulin, 1·5 1·0, and 0·5 per cent. have been employed and the experimental values are graphically represented in Figs. 1 and 2. The correlation between dilatometric depressions and the corresponding fructose values at definite intervals of time is illustrated in Fig. 3, while Table I gives the dilatometric depression in mm^3 per millimol release of fructose.

TABLE I.

Time in mins.	Dilatometric depression (mm^3) per millimol of fructose								
	30	40	60	90	120	150	180	210	240
Inulin 1·5% ..	10·3	9·0	8·5	8·2	8·1	7·7	7·9	7·8	7·6
,, 1·0% ..	8·0	8·0	8·0	8·3	8·1	8·1	7·9	7·8	7·8
,, 0·5% ..	7·6	7·4	7·9	7·6	7·8	7·8	7·8	7·8	7·8

Another set of experiments were carried out employing a slightly different concentration of the enzyme and also the substrate. The kinetics of reaction was not followed but the dilatometric depression and the fructose released after a certain interval of time were estimated. The depression per millimol release of fructose calculated from these data confirm the values given in Table I.

TABLE II.

Concentration of Inulin	Time in mins.	Total depression mm^3	Fructose released mgms.	Depression per millimol fructose mm^3
2·0%	330	25·57	597·9	7·7
1·0%	240	11·49	262·0	7·9
1·0%	240	10·45	241·1	7·8

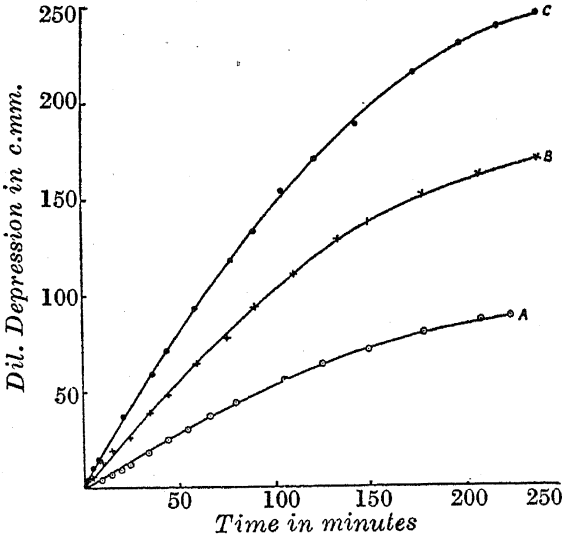

FIG. 1.

A=0·5 % Inulin.
B=1·0 % ,,
C=1·5 % ,,

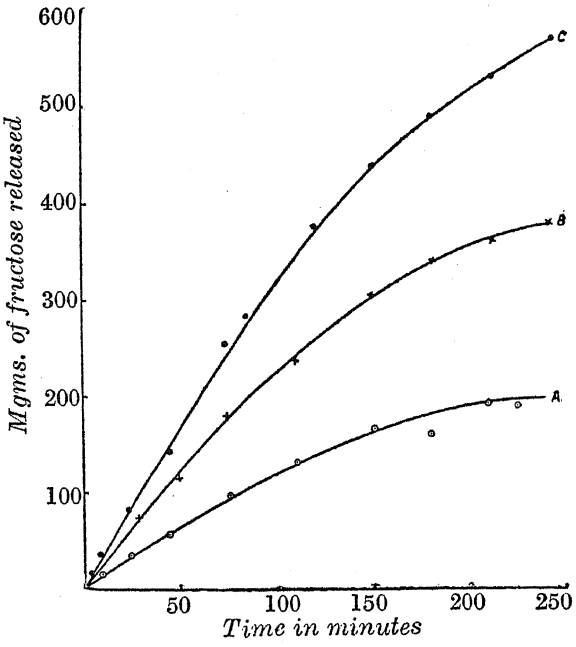

FIG. 2.

A=0·5 % Inulin.
B=1·0 % ,,
C=1·5 % ,,

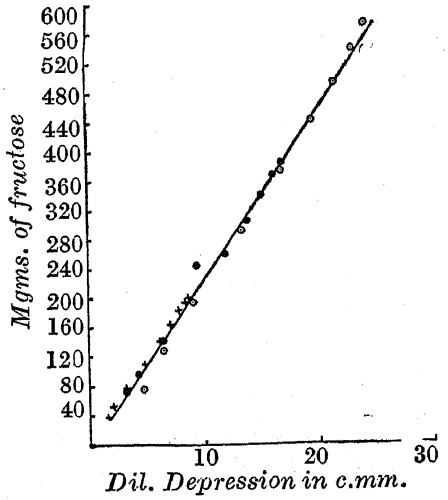

FIG. 3.

⊙ 0·5 % Inulin.
● 1·0 % ,,
+ 1·5 % ,,

Discussion.

It will be seen from the Figs. 1 and 2 that the hydrolysis of inulin can be elegantly followed in the dilatometer. The total depression obtained in the case of inulin hydrolysis for a given concentration is more than twice the value obtained for the hydrolysis of a corresponding concentration of starch (Sreerangachar and Sreenivasaya, 1933).

In general, it may be remarked that any system which involves the release of fructose is accompanied by a greater depression in volume. This view is also supported by values for the hydrolysis of maltose and sucrose which are respectively 3·0 and 6·0 mm^3 (Peter Rona and N. N. Lemmer, 1931 ; Sreenivasaya and Sreerangachar, 1931).

It will be seen from Table I that the values for the dilatometric depression in mm^3 per millimol release of fructose during the first 60 mins. do not show any agreement with the other values obtained for later intervals of time. This is partly due to the large experimental error involved in estimating small quantities (2–20 mgms.) of the released fructose, which by the Bertrand's method can be estimated only with an accuracy of $\pm 0 \cdot 5$ mgm. of sugar. Similarly, since the dilatometric column is measured only with an accuracy of $\pm 0 \cdot 5$ mm., the experimental error involved in measuring small changes (5–20 mm.) in the dilatometric column in the initial stages of the reaction is large. These errors get reduced when larger quantities of fructose have to be estimated and a bigger column of depression has to be measured both of which occur in the later stages of the reaction. Another probable factor contributing to the initial discrepancies may be the disturbance in the water balance of the system as a result of the destruction of the colloidality of the substrate as mentioned before. If, however, the results during the first 40 mins. are not taken into consideration, an average volume depression of 7·9 mm^3 per millimol release of fructose is obtained.

Summary.

1. The kinetics of the enzymic hydrolysis of inulin has been followed in the two-bulbed dilatometer and also by an estimation of the fructose released by Bertrand's method.

2. For a given concentration of substrate, inulin hydrolysis is accompanied by a greater depression of volume than that obtained for starch hydrolysis. It is suggested that systems involving the release of fructose during hydrolysis suffer a greater volume depression ; this is well illustrated by the difference in volume occurring during the hydrolysis of maltose and sucrose.

3. The dilatometric depression per millimol release of fructose during the hydrolysis of inulin is 7·9 mm^3 (average value) and is independent of the concentration of the inulin employed. Errors involved in the early stages of the reaction are indicated.

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