Inhibitory Action of Glucides on Trypsin Activity

GLUCIDES, usually unimportant reagents, can affect the activity of enzymes. Farber and Wynne[1] have reported that some monosaccharides have marked inhibitory effect on the activity of pancreatic proteinase. A similar observation has been made by Whitaker *et al.*[2] with α-amylase. This report is concerned with the inhibitory effect of some mono- and di-saccharides on the activity of trypsin.

Trypsin activity was determined with azocasein substrate, prepared according to Charney and Tomarelli[3]. The trypsin (Merck) was used in 0·1 M phosphate buffer (*p*H 7·5). Glucides were dissolved in the enzyme solution. The reaction mixture, which contained 12·5 mg/ml. azocasein, 1 mg/ml. trypsin, and 1 M (in some cases 0·5 M) glucide, was kept 10 min at 40° C. After precipitating the non-digested proteins with trichloroacetic acid (final concentration 3 per cent), and mixing the filtrate with N sodium hydroxide, the extinction was obtained by means of a Pulfrich photometer with filter *S* 42, using for comparison blanks formed in the same way but without incubation. Trypsin activity was expressed as a percentage of the activity without glucides.

The effect of different concentrations of glucose was first determined (Fig. 1). It may be observed that the inhibitory effect runs parallel with the concentration of glucose. In order to compare the inhibitory effect of different glucides, the following were tested in 1 M concentration: D-arabinose, D-glucose, D-galactose, D-mannose, D-fructose, maltose and saccharose. The two disaccharides were also tested in 0·5 M concentration (Table 1).

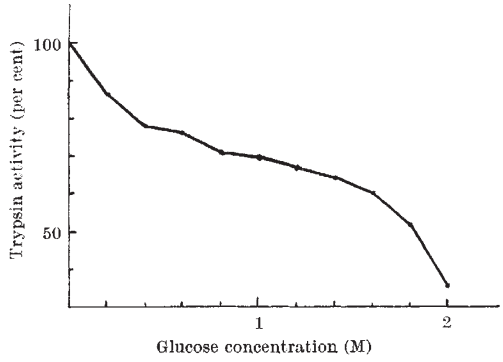

Fig. 1. Change of activity of trypsin with glucose concentration. Incubation time, 10 min; concentration of trypsin 1 mg/ml.; concentration of substrate (azocasein) 12·5 mg/ml. at *p*H 7·5 (in 0·1 M phosphate buffer). Rate of hydrolysis measured photometrically

The strongest inhibitory effect is shown by galactose. In general, except for galactose and arabinose, the inhibitory effect seems to follow the size of the glucidic molecules. These exceptions, as well as the remarkable differences noted in the behaviour of hexoses, suggest, however, that the spatial configuration of the sugars also plays a part in this inhibitory effect. There is no correlation between the inhibitory effect and the solubility of sugars.

The inhibitory effect is principally due to the steric inhibition of the enzymatic reactions, and evidently differs from the well-known substrate inhibition. The glucidic molecules mechanically isolate those of the enzyme and substrate from each other. No doubt, however, the increase of viscosity caused by the sugars also contributes to the decrease of the reaction velocity. The phenomenon is consequently a non-specific retardation rather than a true inhibitory effect, which is non-competitive in type, and which should be called 'retardative inhibition' in order to distinguish it from the other types of inhibition. A similar mechanism plays a part in the protective effect of glucides against the heat inactivation of trypsin[4].

Table 1. INHIBITORY EFFECT OF DIFFERENT GLUCIDES ON TRYPSIN ACTIVITY

Glucide	Concentration of glucide (M)	Trypsin activity %*
D-Arabinose	1	70·8
D-Glucose	1	70·0
D-Galactose	1	54·4
D-Mannose	1	84·3
D-Fructose	1	64·2
Maltose	1	58·8
Maltose	0·5	80·9
Saccharose	1	54·8
Saccharose	0·5	77·1

* Trypsin activity as percentage of activity in the absence of glucides.

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Tumour-promoting and Tumour-inhibiting Fractions from Tumours

RELATIVELY simple preparatory procedures reported from our laboratories[1] have been extended to obtain tumour-promoting fractions (MF_1 and MF_2) and a tumour-inhibiting fraction from lyophilized ascites sarcoma 37 (carried in *BT* mice) and *dbrB* adenocarcinoma (carried in *DBA*/1 mice).

The lyophilized tumour tissue was ground with 4 per cent sodium taurocholate, centrifuged, and the residue treated again with sodium taurocholate. Supernate and residue were combined, shaken, taken to *p*H 7·4 with sodium hydroxide, acidified to *p*H 6·6 with acetic acid, and centrifuged: from this treatment we obtained residue 1 and supernate 1. Residue 1 was washed with sodium taurocholate, extracted twice with acetone, centrifuged, and the final residue, dried *in vacuo*, was fraction MF_1. Supernate 1 was passed through a Seitz filter, the filtrate was brought to *p*H 4·3 with acetic acid, and the precipitate which formed (residue 2) was separated by centrifuging to give supernate 2. Residue 2 was extracted with acetone–water (1 : 1), centrifuged and re-extracted with acetone. The final residue, separated by centrifuging and dried *in vacuo*, was fraction MF_2.

Supernate 2, after Seitz filtration, was adjusted to *p*H 7 with sodium hydroxide and lyophilized. It was then extracted with benzene–ethanol (2 : 1), centrifuged and re-extracted with benzene–alcohol, and the insoluble portion was dried *in vacuo*. It was treated with absolute alcohol, and the soluble supernate separated by centrifuging and evaporated nearly to dryness on a water bath. Addition of ether produced a flocculent white precipitate which was the inhibiting fraction: this was separated by drying *in vacuo*.

Assays of MF_1 and MF_2 fractions from Sarcoma 37 and *dbrB* adenocarcinoma were made in *BT* and *DBA*/1 mice, respectively. The inhibitory fraction was investigated only against the *dbrB* tumour. 0·1 ml. of sarcoma 37 as a 1 : 5 dilution of ascites fluid from a donor was injected subcutaneously into the abdominal region of *BT* mice. The *dbrB* tumour was prepared as a 1 : 1,500 dilution of Type *B* suspension previously described[2] and 0·05 ml. was injected subcutaneously in the abdominal region of *DBA*/1 mice. Five days after tumour inoculation the appropriate fraction was injected intravenously into the tail vein. Fractions MF_1 and MF_2 were homogenized in distilled water (11 mg per ml.) and 0·05 ml. was administered. The inhibiting fraction was given in a 0·1-ml. dosage containing 33 mg per ml. Groups of ten to twelve mice were used for each experiment and control group (which only received the tumour material). Measurements were made of tumour size every 2 days, beginning on the fifth or sixth day after challenge. All control animals