The distribution of trehalase, sucrase, α-amylase, glucoamylase and lactase (β-galactosidase) along the small intestine of five pigs

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1. Sucrase, trehalase (*EC* 3.2.1.28), α-amylase (*EC* 3.2.1.1) and glucoamylase (α-1,4 glucan glucohydrolase, *EC* 3.2.1.3) activities have been measured in the small intestine mucosa of five pigs varying in age from 19–30 weeks. The determinations were made at frequent intervals along the entire length starting 5×10^{-1} m from the pylorus. Lactase (β-galactosidase, *EC* 3.2.1.23) has similarly been measured in one pig.

2. All these enzymes were present in the sample obtained from nearest to the pylorus and rose rapidly in the first few metres.

3. Trehalase and lactase were similarly distributed with a peak activity in the proximal quarter of the small intestine, falling to very low levels in the distal half.

4. Sucrase and glucoamylase resembled one another in distribution pattern with a peak approximately midway along the small intestine, followed by a slight decrease in sucrase activity distally and a rather greater decrease with glucoamylase.

5. α-Amylase activity, assumed to be due to adsorbed pancreatic enzyme, had no regular pattern of distribution.

6. In any individual, the enzyme levels fluctuated considerably along the intestine, with regions where the α-amylase was very high and the levels of the other enzymes were depressed. These regions of high α-amylase activity coincided with regions where the gut was observed to be dilated at dissection.

The presence of two types of amylase in intestine mucosa was demonstrated in the rat by Dahlqvist & Thomson (1963*a*) who identified one of them as a particle-bound enzyme which released only glucose from starch, and was thus an α-1,4 glucan glucohydrolase (glucoamylase, *EC* 3.2.1.3). The other was an α-amylase (*EC* 3.2.1.1) with the characteristics of pancreatic α-amylase. The presence in the small intestine mucosa of amylases of these two types was confirmed in the rat by Ruttloff, Friese & Täufel (1967*a*) and Alpers & Solin (1970). A similar picture was found in the pig by Ruttloff *et al.* (1967*a*), in man by Eggermont (1969) and in the monkey by Seetharam, Swaminanthan & Radhakrishnan (1969, 1970). The distribution of either type of amylase has not been reported in detail in any species.

As already described (Manners & Stevens, 1972), the sucrose hydrolysis by the small intestine mucosa is due to the fact that one of the α-D-glucoside glucohydrolases (α-glucosidase, *EC* 3.2.1.20) has sucrase activity, whereas the lactase activity is due to two or more β-galactosidases (*EC* 3.2.1.23) of different origin and properties. In studies of sucrase and lactase activities along the small intestine of pigs, Manners & Stevens (1972) found considerable variation in sucrase between one individual and another for corresponding positions in the intestine especially in the adult pigs. These

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Table 1. *Details of the experimental pigs*

Identification	Breed	Sex	Age (weeks)	Live weight (kg)	Carcass wt (kg)
83117	Landrace	♀	—	—	49·0
B 2	Large White	♀	19	52·3	—
U 11	Large White	♂	24	77·3	—
MRI 406	Piétrain	♂	—	—	71·0
7536	Landrace	♂	30	112·7	—

variations seemed to be related to the physical condition of the intestine, as observed during disection. Samples, taken in regions where the gut was dilated, consistently had enzyme activities lower than expected. From measurements of hydrolytic activity towards sucrose, lactose and maltose at frequent intervals along the small intestine, Kojecký & Matlocha (1964) obtained irregular distribution curves. To assess the extent to which enzyme levels fluctuate along the small intestinal mucosa, and to study the relation of these fluctuations to the appearance of the gut, the levels of sucrase, trehalase, α-amylase and glucoamylase were measured at twenty-four sites along the small intestine of five pigs. Lactase was also measured in samples from one animal.

MATERIALS AND METHODS

Animals and sampling

Details of the five pigs are given in Table 1. The viscera were removed immediately after slaughter and the small intestines dissected out and sampled as described by Manners & Stevens (1972).

At sampling, notes and photographic records were made of the condition of the small intestine of each animal, with emphasis on any regions of flaccidity and dilatation. The intestinal samples were usually taken at intervals of 5×10^{-1}m but more frequent samples were taken in some dilated regions, particularly where there was a change in gross appearance of the intestine.

Enzyme determinations

Mucosal samples of 100 mg were homogenized in 10 ml cold de-ionized water with a sonic probe for 30 s. Lactase levels were determined by the modified method of Dahlqvist (1964) as described by Manners & Stevens (1972). Sucrase was determined by this method on all the pigs except no. 83117.

Sucrase was also determined on all five pigs using a technique which depends on reduction of alkaline ferricyanide by the reducing sugars, produced on hydrolysis (Kidder, Hill & Stevens, 1972). This method was also used for trehalase and the amylases. To distinguish between the α-amylase and the glucoamylase, a portion of the homogenate was mixed with 0·1 M-EDTA in pH 6 phosphate buffer in the ratio 20:1 (v/v), and heated at 50° for 15 min. This inactivates the α-amylase but does not affect the glucoamylase (Ruttloff *et al.* 1967*a*). The amylase activity of the heated homogenate then represented the glucoamylase and the difference between this and
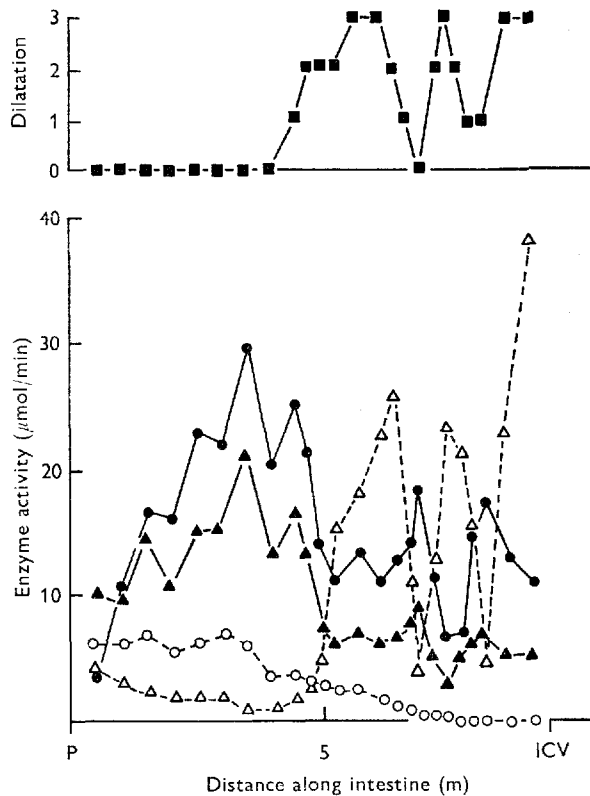
Fig. 1. Mucosal enzyme activity and degree of dilatation along the small intestine of pig 83117. Upper scale: state of intestine, turgid (o) slightly dilated (1) partly dilated (2) or fully dilated (3). Lower scale: activity per g mucosa of trehalase (○), sucrase (●), α-amylase (△) and gluco-amylase (▲) along the intestine from the pylorus (P) to the ileo-caecal valve (ICV).

the activity of the unheated homogenate was presumed to be due to the α-amylase. All the enzyme activities were determined at pH 6 using phosphate buffer and expressed for the disaccharidases as μmol substrate split per g mucosa per min, and for the amylases as μmol reducing sugar produced per g mucosa per min.

RESULTS

Enzyme activity

The sucrase values determined by the two methods showed satisfactory correspondence, but the results with the ferricyanide reduction method were about 24% higher than those by the Dahlqvist method. As only the ferricyanide reduction method was used for all five pigs, the results given are those obtained by this method.

Figs. 1–5 show the levels of enzyme activity found in each of the five pigs and the location of the dilated areas found at dissection. The distribution of sucrase and lactase activities resembled that previously reported (Manners & Stevens, 1972), namely low lactase activity which was almost entirely confined to the proximal half of the small
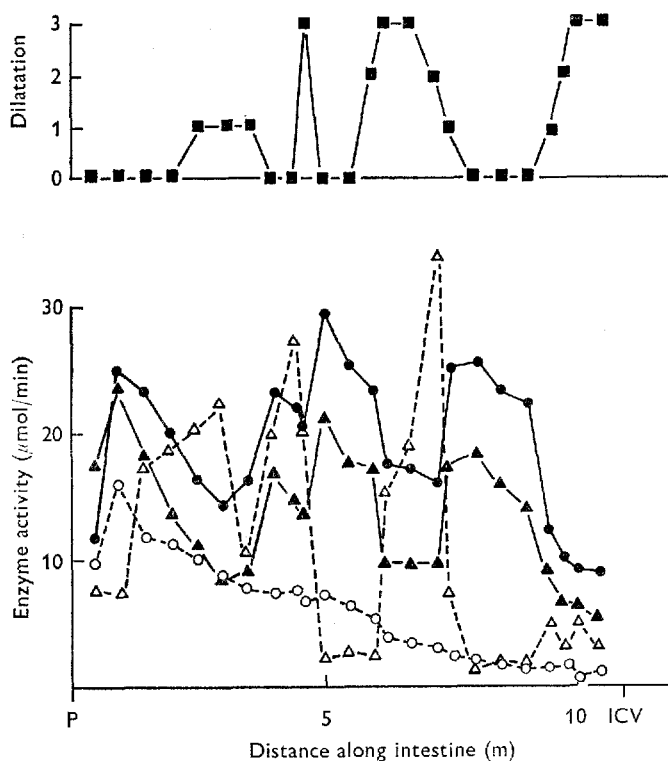
Fig. 2. Mucosal enzyme activity and degree of dilatation along the small intestine of pig B 2. Symbols as in Fig. 1.

intestine, and much higher levels of sucrase with a peak towards the middle of the intestine. Trehalase levels followed a pattern similar to that of lactase with a peak in the proximal jejunum. Glucoamylase activity had the same distribution as sucrase, although with a lower glucoamylase:sucrase ratio in the distal small intestine.

Sampling at frequent intervals showed that all four of these activities, particularly sucrase and glucoamylase, are subject to considerable fluctuations in activity along the intestine. These fluctuations were normally found to be similar in these four carbohydrases, although varying in extent.

Dilatation of the intestine

Flaccid, distended regions of the small intestine were noted in all the pigs. The intestine of pig U 11 (Pl. 1) is typical. The criteria for describing regions as flaccid or dilated were loss of muscular tone, notable increase in both external and internal circumference and reduction in mucosal folding. There was also brown or yellow discoloration of the mucosa, accumulation of mucus and retention of ingesta (liquid, gas and coarse solid material). Badly distended areas often had a foul smell resembling hydrogen sulphide. This picture contrasts with what we have called the 'normal' condition of the small intestine when examined freshly *post mortem*, which is turgid,
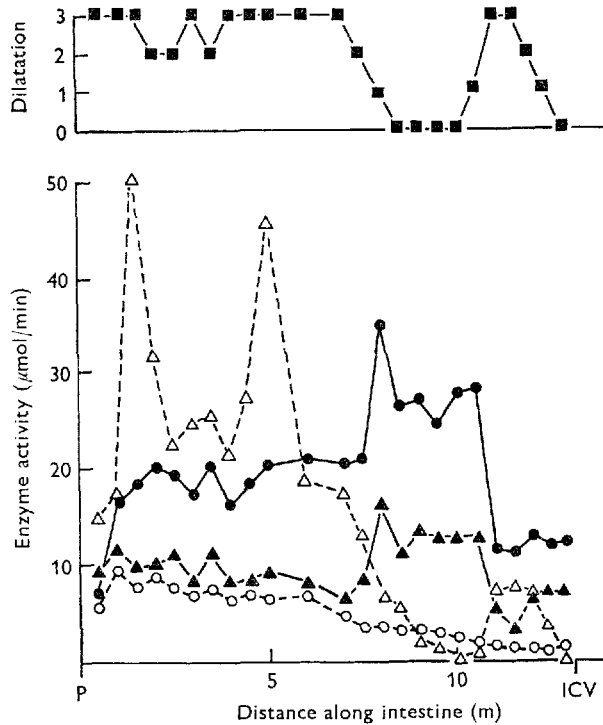
Fig. 3. Mucosal enzyme activity and degree of dilatation along the small intestine of pig U 11. Symbols as in Fig. 1.

has well-marked mucosal folds, a closed lumen and presents a clean, pinkish appearance. The dilated areas usually correspond with regions of high α-amylase activity and lowered activity of the other enzymes (Figs. 1–5).

DISCUSSION

The marked irregularity of distribution along the small intestine of the levels of all the enzymes agrees with the findings of Kojecký & Matlocha (1964), who found similar fluctuations when they determined hydrolytic activity towards sucrose, lactose and maltose at 7.5×10^{-1} m intervals along the small intestine of two pigs. This irregularity would account for the disagreement between various authors (Dahlqvist, 1961; Kojecký & Matlocha, 1964; Manners & Stevens, 1972) on the location of peak sucrase activity and would also account for the variability of sucrase activity found by Manners & Stevens (1972) at six widely spaced sites in the small intestine.

Sucrase, trehalase and lactase

The distribution of sucrase and lactase activity was consistent with the findings of Manners & Stevens (1972). The location of maximum trehalase activity was in the proximal part of the small intestine, with its distribution resembling that of lactase. This agrees with the results of Dahlqvist (1961) in a pig, of Dahlqvist & Thomson
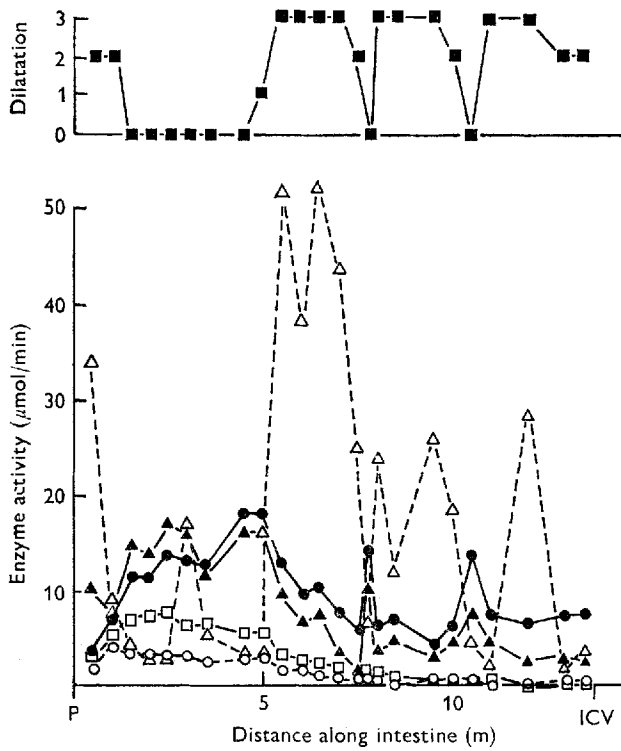
Fig. 4. Mucosal enzyme levels and degree of dilatation along the small intestine of pig MRI 406.
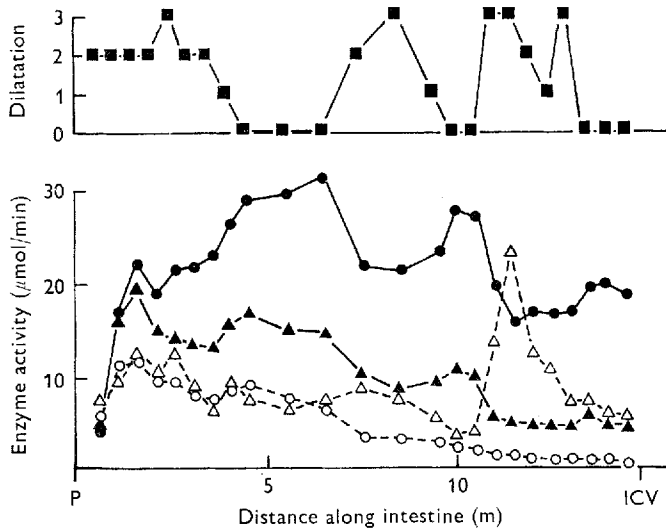Lactase levels (□), other symbols as in Fig. 1.

Fig. 5. Mucosal enzyme levels and degree of dilatation along the small intestine of pig 7536.
Symbols as in Fig. 1.

(1963*b*) and Rubino, Zimbalatti & Auricchio (1964) in the rat, of Siddons (1968) in the bovine and Madge (1970) in the mouse. The sucrase activities were, in the whole, higher than those reported when six samples were taken from each of a larger group of pigs of similar age (Manners & Stevens, 1972). This is mainly due to the method used in our experiment, which gave sucrase values about 24% higher than those obtained by the modified Dahlqvist method. In addition, there is considerable individual variation which is more noticeable when a large number of samples is taken. The positions with the highest sucrase activities in this experiment might well have been missed if only six samples had been taken.

Glucoamylase

The brush-border glucoamylase activity is due to one or more of the α-glucosidases which Dahlqvist (1962) classified as 'maltases' Ia, Ib, II and III. The 'maltase Ia' is responsible for almost all the hydrolytic activity of the intestine mucosa towards iso-maltose, and so is additionally an oligo-1,6-glucosidase (oligodextrin-6-glucano-hydrolase, *EC* 3.2.1.10), whereas the 'maltase Ib' is responsible for all the hydrolytic activity in the intestine mucosa towards sucrose. Dahlqvist & Thomson (1963*a*) in rats, and Eggermont (1969) in man, showed that the mucosal glucoamylase activity was separable from the hydrolytic activity towards isomaltose and sucrose, and was a property of an α-glucosidase fraction relatively stable to heat. Kidder *et al.* (1972) using pig mucosa found that at least two fractions of different heat-stability appeared to be present. It is thus probable that the glucoamylase activity in small intestine mucosa is due to the enzymes which Dahlqvist (1962) classified as 'maltase II' and 'maltase III'.

The distribution of glucoamylase showed a striking similarity to that of sucrose but the ratio of sucrase:glucoamylase activity was always greater at the distal end of the small intestine. Eggermont & Hers (1969) postulated a 1:1:1 molecular association in human small intestine mucosa of sucrase, glucoamylase and oligo-1,6-glucosidase. This would not be consistent with our results in the pig, if all the glucoamylase activity is due to the same enzyme, but would be possible if it is due to two or more enzymes with glucoamylase activities of different intensities and different distributions along the gut. It would, however, not be consistent with the views of most other workers, who found a 1:1 association of sucrase and oligo-1,6-glucosidase (Kolínská & Semenza, 1967; Dahlqvist & Telenius, 1968) but found this complex to be quite separate from any other α-glucosidases.

α-Amylase

The pancreatic origin of mucosal α-amylase has been demonstrated by Alpers & Solin (1970), who showed that this enzyme was not present in the mucosa of rats in which the pancreatic juice had been surgically diverted from the intestine, but could be restored by exposing the mucosa to pancreatic juice in vitro. Hill (1971) and Kidder *et al.* (1972) found this enzyme to be absent from the small intestine mucosa of dogs which had suffered from pancreatic degenerative atrophy but present there at a high level in normal dogs.

Ugolev (1960), who first demonstrated the adsorption of pancreatic α-amylase on

small intestine mucosa in experiments on rat mucosa in vitro, postulated that it was important in vivo. He stated (Ugolev, 1965) that, in the rat, the adsorbed pancreatic amylase provided half of the total amylase activity of the mucosa. Ruttloff, Friese & Täufel (1967b) and Ruttloff, Friese, Noack & Täufel (1968) showed that the α-amylase in pig and rat small intestine mucosa had much the greater action on soluble starch, whereas the glucoamylase had the greater effect on the smaller dextrins.

Nothing had previously been reported on the distribution of adsorbed pancreatic amylase along the intestine beyond the observation of Ugolev (1960) that the capacity to absorb this enzyme was a characteristic of the small intestine mucosa but of no other part of the alimentary tract. It is clear from our results that, in the pig, this adsorptive capacity is very much greater in some regions than in others, and is determined, not so much by the distance along the small intestine as by the condition of the intestine in the particular locality. A relationship between adsorption of α-amylase and regions of dilatation in the intestine has been shown, but it may be that the important factor in this relationship is the presence of increased amounts of mucus in these regions. de Laey (1966, 1967), in observations on the occurrence of α-amylase in the small intestine, has reported that the enzyme binds to mucin.

The inverse relationship between high α-amylase activity and reduced levels of the other carbohydrates suggests either mucosal damage or a substantially smaller cellular content in the mucosal samples of these regions. de Lacy (1966, 1967) reported lower activity of adsorbed α-amylase associated with deterioration of mucosal structure, but in our observations the mucosa appeared to be in better condition in those regions with low α-amylase activity.

The introduction of enteropathogenic strains of *Escherichia coli* into the lumen is known to cause dilatation of intestinal loops (De, Bhattacharya & Sarkar, 1956). The phenomenon has been demonstrated in the pig with both the organisms and their secretion products (Moon, Sorensen & Sautter, 1966; Smith & Halls, 1967a, b; Gyles & Barnum, 1967), and the presence of such organisms attached to the mucosa of pig small intestine has been demonstrated by Arbuckle (1970, 1971). The failure of a dilated region to achieve complete closure and expulsion of contents during peristalsis could help to maintain an increased bacterial population. A self-perpetuating situation can be visualized in which a locally high population of certain types of bacteria maintains a degree of dilatation which, in turn, helps to maintain this bacterial population.

Plate 1

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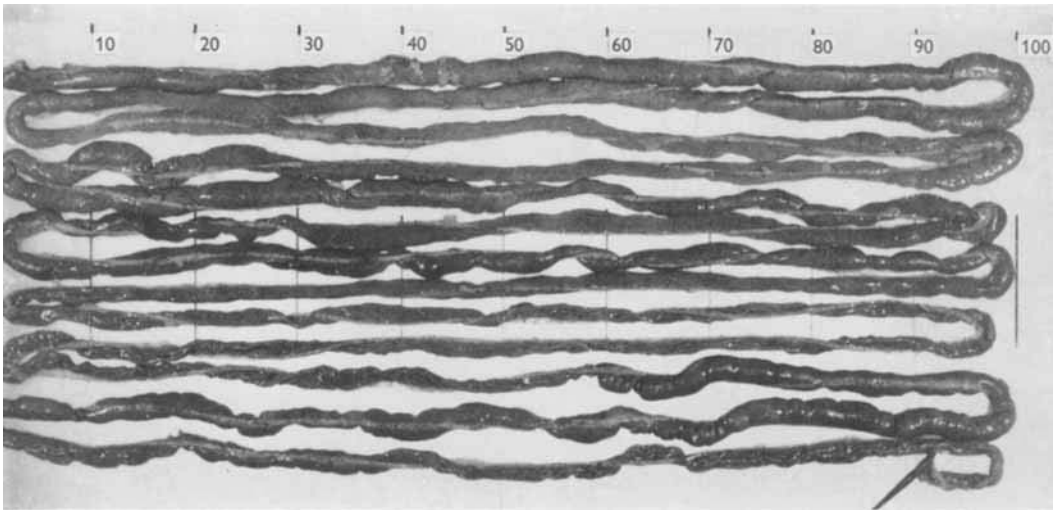
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EXPLANATION OF PLATE I

The small intestine of pig U 11, showing flaccid distended regions. The scale is in 10^{-2} m.