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The Location of Carbohydrases in the Digestive Tract of the Pig

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Little is known about the locations in the mammalian intestine at which hydrolysis of disaccharides and polysaccharides occurs.

In a recent investigation of intestinal digestion and absorption in man, Borgström, Dahlqvist, Lundh & Sjövall (1957) observed that the invertase activity (in unpublished experiments we have demonstrated that the maltase and lactase activities are distributed similarly) of the intestinal contents, which was weak throughout the whole small intestine, was maximal in the lower part of the small intestine (lower jejunum and ileum). In spite of this, the absorption of lactose occurred in the upper part of the small intestine (duodenum and upper jejunum), where no disaccharidase activity could be demonstrated in the intestinal contents (Ammon & Henning, 1956; Borgström *et al.* 1957). The hydrolysis of disaccharides during their absorption is catalysed by enzymes apparently situated inside the cells of the intestinal mucosa (Borgström *et al.* 1957). Little information is, however, available about the relative disaccharidase activity of the mucosa of different parts of the small intestine.

Amylase, in contrast to the disaccharidases, is mainly secreted into the intestinal lumen in the

pancreatic juice. However, preparations from the intestinal mucosa also contain amylase. Intestinal-mucosa preparations from different species of mammals have been reported to contain one further polysaccharidase, namely dextranase (Adrouny, Bloom & Wilhelmi, 1957). It does not seem to be known, however, whether dextranase is present in the pancreas too.

This paper records the carbohydrase activities of homogenates of mucosa from different parts of the small intestine, from the stomach and from the colon, and of a homogenate of pancreatic tissue. The adult pig was selected as an experimental animal.

EXPERIMENTAL

Determination of enzymic activities

Disaccharidase activities. These were measured by the methods described previously (Dahlqvist, 1960*d*). One unit of disaccharidase activity causes 5% of hydrolysis of the particular disaccharide in 2·0 ml. of reaction mixture at 28 mM-substrate concentration in 60 min. at 37°.

Amylase activity. The substrate solution was prepared by dissolving 2·0 g. of soluble starch a.m. Zulkowsky (from Merck A.G., Germany) and 40 mg. of NaCl in 0·05 M-phosphate buffer, pH 6·9 (3·026 g. of KH_2PO_4 and 3·959 g. of $Na_2HPO_4,2H_2O$/l.), to a final volume of 100 ml. Toluene (1 ml.) was added as a preservative and the solution was stored in a refrigerator. The substrate solution was prepared weekly.

For determination of amylase activity, 1·0 ml. of suitably diluted enzyme solution was mixed with 1·0 ml. of substrate solution and immersed in a water bath at 37°. After 60 min. the reaction was interrupted by the addition of 2·0 ml. of dinitrosalicylate reagent (prepared as described by Hostettler, Borel & Deuel, 1951). A blank was prepared with the same composition, in which, however, the 3:5-dinitrosalicylate reagent was added immediately after the mixing of the enzyme and substrate. The tubes were immersed in a boiling-water bath for 10 min. and then chilled for 2 min. with running tap water. After dilution with 20·0 ml. of water the intensity of the red colour produced was measured in a Beckman B spectrophotometer at a wavelength of 530 mμ, in 1 cm. cuvettes. A standard curve was prepared from known solutions of maltose (the tubes containing 0·5–2·0 mg. of maltose monohydrate). One unit of amylase activity causes an increase of reducing power corresponding to 1 mg. of maltose monohydrate in 60 min. during these conditions. If the increase of reducing power does not exceed that of 2 mg. of maltose monohydrate, the enzymic reaction follows zero-order kinetics and the amount of reducing groups liberated is proportional to the amount of enzyme present.

The amylase unit, when defined in this way, is comparable with the unit used for disaccharidase activity (Dahlqvist, 1960*d*). It should not, however, be confused with the amylase unit used by Borgström *et al.* (1957), since in that case the incubation was performed for 3 min. at 25°.

Dextranase activity. The substrate solution for determinations of dextranase activity was prepared by dissolving 2·0 g. of dextran (dextran 40, mol.wt. by light-scattering 41 000, by end-group analysis 26 000, obtained from Pharmacia A.B., Sweden) in 0·1 M-maleate buffer (Gomori, 1955), pH 6·0, to a final volume of 100 ml. Incubation and determination of the degree of hydrolysis were performed in exactly the same way as for determinations of amylase activity. Maltose was used for preparation of the standard curve, since maltose and isomaltose have the same extinction coefficient with the 3:5-dinitrosalicylate reagent. One unit of dextranase activity is the amount of enzyme which causes an increase of reducing power equal to that of 1 mg. of maltose monohydrate in 60 min.

Determination of protein

The method of Lowry, Rosebrough, Farr & Randall (1951) was employed, the modified reagent B introduced by Eggstein & Kreutz (1955) being used. A standard curve was prepared with human serum albumin (kindly supplied by A. B. Kabi, Sweden).

Preparations of homogenates

The stomach, small intestine, upper part of the colon and pancreas of an adult pig were cut out immediately after slaughter and chilled with crushed ice during transport to the laboratory. From pieces of stomach, small intestine and colon the mucosa was scraped off with a glass slide and homogenized in an Ultra-Turrax homogenizer for 2 min. with an equal weight of 0·9% NaCl. A piece of the pancreas was homogenized in the same way. This method has earlier been found suitable for the extraction of glycosidases from hog small-intestinal mucosa (Borgström & Dahlqvist, 1958). After centrifuging in a Wifug laboratory centrifuge for 5 min., the opalescent supernatant was assayed for carbohydrase activities.

RESULTS

There are several ways of expressing the relative carbohydrase activities of the different segments of the intestine. Some authors have expressed the activity per cm.^{2} of intestine (Euler & Svanberg, 1921). Because of the enormous surface area of intestinal villi, as well as their variation in density along the length of the intestine, it seems more logical to compare the activity per gram of mucosa (Cajori, 1935; Heilskov, 1951). Since in the present investigation the homogenates have always been prepared from a mixture of equal weights of mucosa and 0·9% NaCl, the carbohydrase activities can be compared directly when expressed per ml. of homogenate preparations.

The protein content of the homogenates varied (Table 1), however, and therefore in Figs. 1–5 the specific carbohydrase activity (i.e. number of units/mg. of protein) has been used to enable comparison of the relative carbohydrase activities of the different segments of the small intestine. However, the results are similar whether enzyme activity is presented as units/mg. of mucosal protein or units/ml. of mucosal homogenate. The experimental conditions for carbohydrase assay were such that 0·5 unit/ml. of each of the activities investigated could be detected.

To check the completeness of the removal of the mucosa with the glass slide, in one experiment the remaining intestinal wall was also homogenized, and the homogenate was assayed for invertase activity. It was found that 80% of the total amount of invertase originally present in the piece of intestine had been removed with the mucosa.

Stomach. No invertase, maltase, isomaltase, trehalase, lactase, cellobiase or dextranase activity could be detected. The preparation had very weak amylase activity (17 units/ml.; cf. below), which may very well have been caused by contamination with saliva from the gastric contents. The mucosa of the stomach therefore does not seem to contain any carbohydrases.

Small intestine. This had a total length of 16 m. The duodenum had a length of 0·34 m., the jejunum and the ileum had each a length of about 8 m. In the duodenum and jejunum no intestinal contents had to be removed, but in the ileum the contents were removed and the intestine was gently blotted with a piece of cloth before the mucosa was scraped off. The pieces of the small intestine selected are noted in Figs. 1–5. The amount of mucosa obtained from 50 cm. of small intestine varied between 7 and 11 g.

Table 1. *Protein content of the homogenates*

All tissues were homogenized with one part (v/w) of 0·9% NaCl soln.

Source of homogenate	Protein (mg./ml.)
Stomach	11·5
Duodenum	56·0
Upper jejunum	63·0
Lower jejunum	41·0
Upper ileum	30·0
Lower ileum	34·0
Colon	25·0
Pancreas	45·0

Invertase activity was low in the duodenum (7·5 units/ml. of homogenate) and was greatest in the lower jejunum and the ileum (50–70 units/ml.) (Fig. 1). Even the homogenate prepared from a section of the lower ileum, cut just proximal to the ileocoecal valve, had strong invertase activity (54 units/ml.).

Maltase activity of the homogenate from the duodenum was 66 units/ml., which is nearly 10 times its invertase activity. The maltase activity also increased in the lower part of the small intestine (Fig. 1) but not to the same extent as the invertase activity. In the homogenates from the lower jejunum and the ileum the maltase activity was 100–150 units/ml., which is two to three times the invertase activity.

The maltase activity of the small intestine of the pig has recently been demonstrated to be effected by a mixture of three different maltases (maltase I–III). The results of the determination of each of these enzymes, by the methods described earlier (Dahlqvist, 1959, 1960*c*, *d*), with 28 mM-substrate

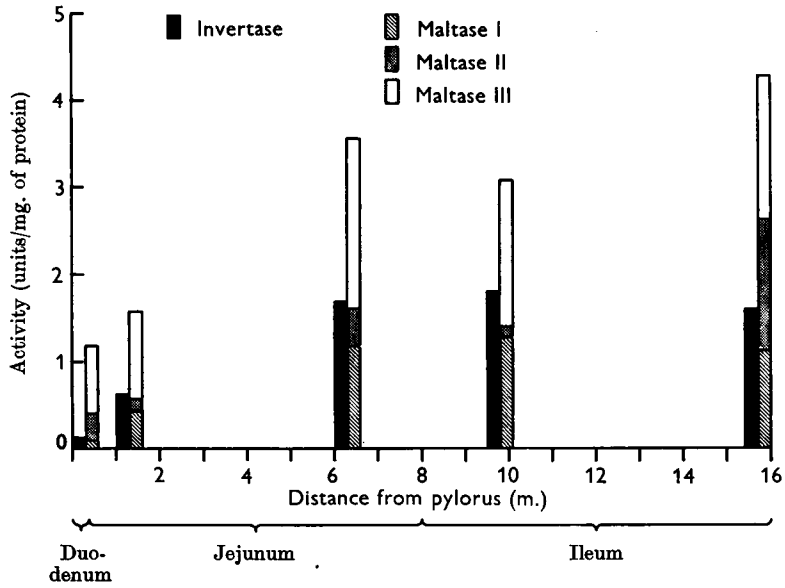

Fig. 1. Invertase and maltase activities of homogenates of mucosa from different parts of the small intestine of an adult pig. The three different maltases (maltase I–III) present in such preparations have been measured separately by the methods described earlier (Dahlqvist, 1959, 1960*c*, *d*).

concentration (Dahlqvist, 1960*d*), are seen in Fig. 1. Maltase II activity, as compared with maltase III, is unusually low. Other experiments with homogenates prepared from the small intestine of the pig have demonstrated that maltase II activity is usually equal to or somewhat greater than maltase III activity; maltase II and maltase III together usually exert 80–85 % of the total activity (Dahlqvist, 1960*d*).

Isomaltase activity, which is to some extent due to the presence of maltase II and maltase III but mostly (75 %) to a 'specific' isomaltase (Dahlqvist, 1960*b*), had a distribution in the small intestine which was similar to that of the invertase and maltase activities (Fig. 2), i.e. the isomaltase activity per ml. of homogenate rose from a low value (1·5 units/ml.) in the preparation from the duodenum to about 30 units/ml. in the homogenates from the lower jejunum and the ileum.

Trehalase activity, which is due to a specific enzyme (Dahlqvist, 1960*a*), showed a distribution which was quite opposite to that of the enzymes described above (Fig. 3). The trehalase activity was greatest in the preparations from the duodenum and the jejunum (15–20 units/ml. of homogenate) but decreased in the lower part of the small intestine.

Lactase activity was greater in the homogenate from the upper jejunum (18 units/ml.) than in that from the duodenum (9 units/ml.), but was practically absent from the homogenates of the ileum (Fig. 4). The cellobiase activity had the same distribution as the lactase activity, and the ratio of cellobiase to lactase was the same in homogenates from all parts of the small intestine (Fig. 4). This suggests the possibility that the cellobiase and lactase activities are caused by one and the same enzyme. The relation between these two activities is under investigation in our laboratory.

Amylase activity was present in all the homogenates of small-intestinal mucosa, but was low (200–700 units/ml.) when compared with the activity in the homogenate of the pancreas (see below). It is quite possible that at least some of the activity of the mucosa homogenate was caused by contamination with intestinal contents, which contain considerable amounts of pancreatic amylase.

The amylase activity of the homogenates, prepared from different parts along the small intestine, varied irregularly.

Dextranase activity was low in all the homogenates, but increased in a distal direction in the small intestine from 1 unit/ml. in the homogenate from the duodenum to 5·5 units/ml. in that from the lower ileum (Fig. 5). Since the homogenate of the pancreas had no dextranase activity (see below) the activity of the homogenates of intestinal mucosa could not be caused by contamination with pancreatic juice. The low dextranase activity of the samples, together with the localization of this activity to the distal part of the small intestine, might suggest that the dextranase activity was

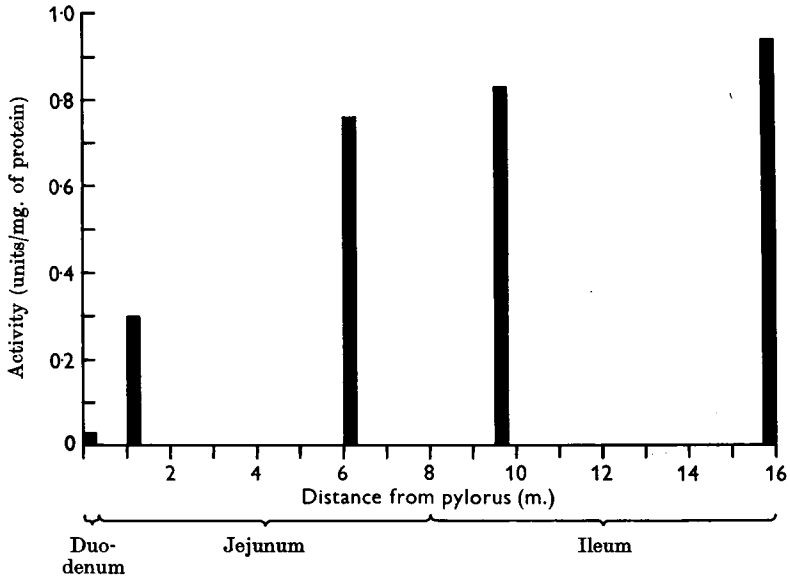

Fig. 2. Total isomaltase activity of homogenates of mucosa from different parts of the small intestine of an adult pig. The isomaltase activity of such preparations is exerted by a mixture of three different enzymes as described previously (Dahlqvist, 1960*b*).

caused by contamination with intestinal bacteria. Against this possibility is, however, the low dextranase activity of the homogenate of mucosa from the colon (Table 2).

Colon. The mucosa from the colon was scraped off a few centimetres distal to the ileocoecal valve. The carbohydrase activities of the homogenate are seen in Table 2. Although most of the carbohydrase activities were present in this preparation, too, they were present only in small amounts, as compared with the activities of the homogenates of small intestinal mucosa.

Pancreas. The homogenate of the pancreas was kept chilled with crushed ice until just before assay, and all assays were completed within a few hours after the homogenization.

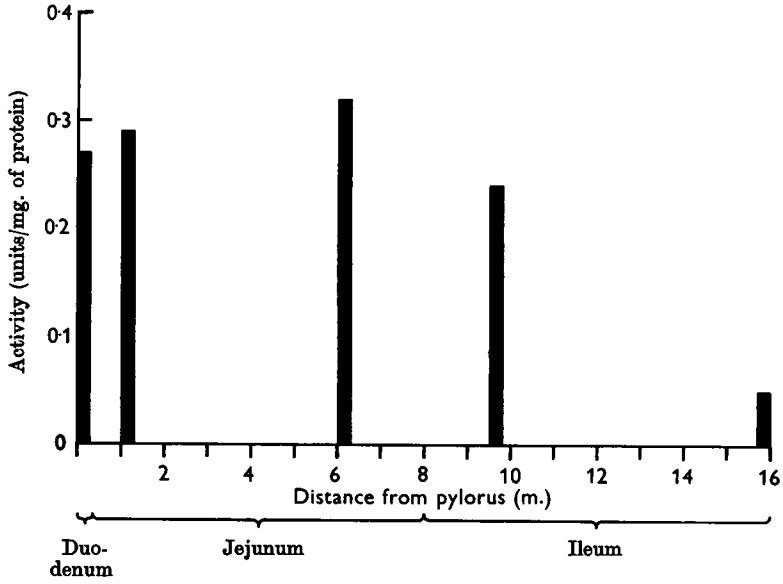

Fig. 3. Trehalase activity of homogenates of mucosa from different parts of the small intestine of an adult pig.

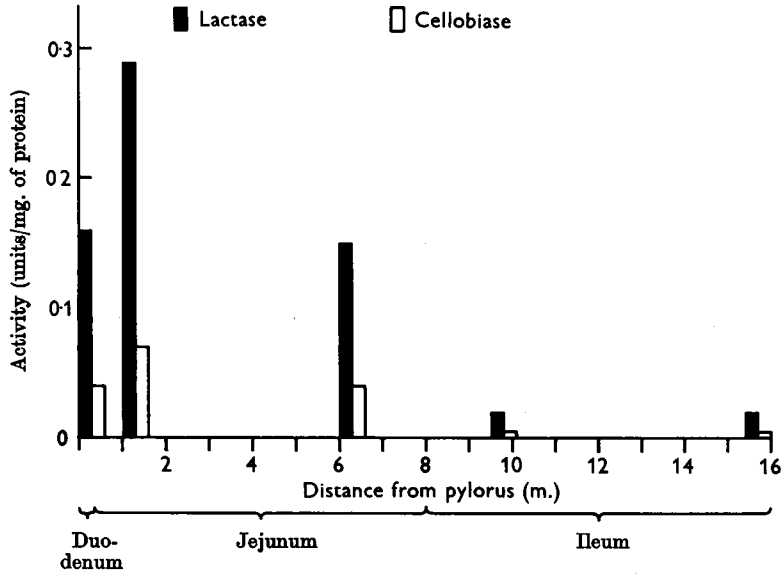

Fig. 4. Lactase and cellobiase activities of homogenates of mucosa from different parts of the small intestine of an adult pig.

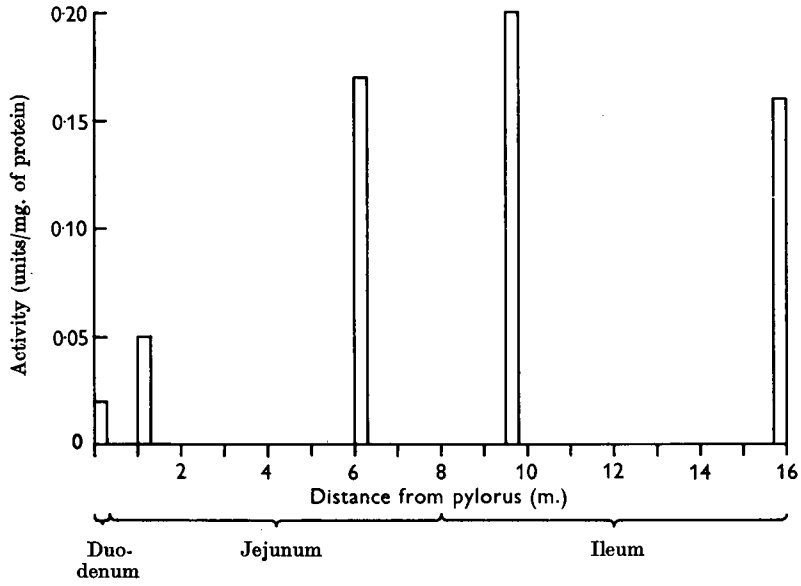

Fig. 5. Dextranase activity of homogenates of mucosa from different parts of the small intestine of an adult pig.

Table 2. *Carbohydrase activities of the homogenate of mucosa from the colon of an adult pig*

Protein content of the homogenate was 25 mg./ml.

Enzyme	Activity (units/ml. of homogenate)
Invertase	8
Maltase I	6
Maltase II	18
Maltase III	0
Isomaltase	2
Trehalase	0·5
Lactase	0
Cellobiase	0
Amylase	250
Dextranase	1

Only two carbohydrase activities were recognized, namely very active amylase (80 000 units/ml.) and weak maltase (12 units/ml.) activity. This amylase activity was more than 100 times that of the homogenate of small-intestinal mucosa, which suggests that it is mainly pancreatic amylase which effects the hydrolysis of starch in the small intestine.

The pancreatic-maltase activity, by contrast, was very weak, whether compared with that of the preparations of small-intestinal mucosa (see above) or with the pancreatic-amylase activity. It may therefore be concluded that the pancreatic maltase is without physiological importance for the hydrolysis of maltose in the small intestine.

DISCUSSION

It is apparent that the disaccharidases are mainly localized in the small intestinal mucosa. The weak maltase activity of the pancreas and the weak disaccharidase activities of the mucosa of the colon cannot contribute to any considerable extent to the hydrolysis of ingested disaccharides.

The findings about the location of the disaccharidase activities along the small intestine seem remarkable. From the present investigation it appears that there exist two major groups of pig-intestinal disaccharidase activities, according to their distribution along the small intestine. One group (invertase, maltase and isomaltase) is localized mainly in the distal part of the small intestine, and the other group (trehalase, lactase and cellobiase) is localized mainly in the proximal part of the small intestine. It seems tempting to conclude that the corresponding disaccharides are hydrolysed and absorbed in the corresponding parts of the small intestine. Our previous finding that lactose is absorbed in the proximal part of the small intestine of humans (Borgström *et al.* 1957) agrees with this idea, but further experimental studies are required.

The location of the invertase activity differs from the findings by some earlier authors who have studied other species. Euler & Svanberg (1921) analysed a human small intestine and found that the invertase activity (per cm.^{2} of intestine) was highest in the upper part of the jejunum, whereas

the lower ileum had only 5% of this activity. Recently Ammon & Henning (1956) have found a similar distribution of invertase in rabbits. In the present paper figures are reported for one pig only. Experiments with pieces of intestine of several other pigs have, however, demonstrated that there are hardly any individual variations in the location of the different carbohydrases.

The location of the lactase activity along the small intestine agrees well with that given in earlier reports. Cajori (1935) has found that the lactase activity of mucosa preparations from dog small intestine was 30% greater in preparations from the jejunum than in those from the duodenum (calculated per gram of mucosa), and Heilskov (1951) has found that the lactase activity of the mucosa in different mammals (rabbits, cows and human foetuses) diminishes in the distal segments of the small intestine.

That the homogenate of the pancreas contained a powerful amylase but no disaccharidases, except a very weak maltase, is in accordance with earlier reports, which state that the pancreas contains amylase and maltase, but no invertase (Brown & Heron, 1880; Oppenheimer, 1925), lactase (Heilskov, 1951) or trehalase (Frèrejacque, 1953). The absence of disaccharidase activities from the pancreatic juice can also be deduced from the finding that the small-intestinal contents contain amylase but essentially no disaccharidases (Ammon & Henning, 1956; Borgström *et al.* 1957).

The fact that the homogenate of the pancreas had no dextranase activity demonstrates that intestinal dextranase is a specific enzyme, distinguished from amylase. Like the disaccharidases the dextranase is located in the intestinal mucosa.

SUMMARY

1. The carbohydrase activities of homogenates prepared from mucosa of the stomach, small intestine and colon, and from pancreatic tissue, of an adult pig have been studied.

2. The preparation from the stomach did not contain any carbohydrases except a very weak amylase activity, which may have been caused by contamination with saliva from the gastric contents.

3. The preparations from the small intestine had powerful disaccharidase activities which showed different locations along the small intestine: one group of activities (invertase, maltase and isomaltase) was mainly localized in the distal part of the small intestine, and another group (trehalase, lactase and cellobiase) was localized in the proximal part. This may indicate that different disaccharides are absorbed in different parts of the small intestine.

4. The preparation from the small intestine also had amylase activity, which, however, was low compared with that of the pancreas homogenate and may have been due to contamination with pancreatic juice.

5. Dextranase activity was present in the preparations from the small intestine, but not in those from the pancreas. The dextranase activity is thus caused by a specific enzyme which has its origin in the small intestinal mucosa.

6. The preparation from the colon also had carbohydrase activities, although these activities were weak compared with those of the small intestinal mucosa.

7. The preparation from the pancreas had very powerful amylase activity, and the pancreas seems to be the main source of the amylase of the intestinal contents. This preparation also had weak maltase activity. The preparation from the pancreas had no other disaccharidase activities.

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