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Carbohydrase Activities in the Bovine Digestive Tract

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1. The carbohydrase activities of homogenates of mucosa from the abomasum, small intestine, caecum and colon, and of the pancreas of cattle were studied. 2. The disaccharidase activities were located mainly in the small intestine and showed a non-uniform pattern of distribution along the small intestine; trehalase activity was highest in the proximal part, lactase and cellobiase activities were highest in the proximal and middle parts and maltase activity was highest in the distal part. 3. The intestinal lactase and cellobiase activities were highest in the young calf and decreased with age, whereas the intestinal maltase and trehalase activities, which were very low compared with the lactase activity, did not change with age. 4. No intestinal sucrase or palatinase activity was detected in the calf or in the adult cow. 5. Homogenates of intestinal mucosa also exhibited amylase and dextranase activity. 6. Homogenates of the pancreas possessed a strong amylase activity and a weak maltase activity. The maltase activity did not change with age, whereas the amylase activity increased with age. 7. No marked differences were observed between the carbohydrase activities of calves fed solely on milk and those of calves given a concentrate–hay diet from 6 weeks of age.

Carbohydrate utilization in the calf has been studied in digestion trials (Larsen, Stoddard, Jacobson & Allen, 1956; Huber, Jacobson, McGilliard, Morrill & Allen, 1961*b*; Morrill, Jacobson, McGilliard & Hotchkiss, 1965) and by measuring the response in the blood sugar concentration to the ingestion of various carbohydrates (Dollar & Porter, 1957, 1959; Velu, Kendall & Gardner, 1960; Huber, Jacobson, McGilliard & Allen, 1961*a*). These studies have shown that lactose utilization is very rapid in young calves and decreases with age, whereas maltose utilization is poor in young calves and subsequently increases. The extent to which starch and sucrose are utilized by the calf is unclear because, though digestion trials have indicated a measurable utilization of both carbohydrates, no increase in blood reducing sugar occurs after feeding with these carbohydrates. Some workers (Huber *et al.* 1961*b*; Dollar & Porter, 1957) have suggested that the disappearance of starch and sucrose observed in digestion trials might be due to the action of micro-organisms in the posterior part of the digestive tract.

The ability to utilize dietary disaccharides and polysaccharides is closely correlated with intestinal carbohydrase activity (e.g. Deren, Broitman & Zamcheck, 1967; Paes, Searl, Rubert & Faloon, 1967; Hembry, Bell & Hall, 1967; Kretchmer & Sunshine, 1967; Rubino, Zimbalatti & Auricchio, 1964; London, Cuatrecasas, Birge & Segal, 1967). Thus a quantitative study of intestinal carbohydrase activities is useful in assessing the ability of an animal to utilize various carbohydrates. The present study was undertaken to make a detailed investigation of the carbohydrase activities of homogenates of mucosa from different parts of the digestive tract and of the pancreas of the young calf, and to determine the effect of age on these activities.

MATERIALS AND METHODS

Preparation of homogenates. Guernsey bull calves, which had been maintained on whole-milk diets, were killed at selected ages by intravenously injecting an overdose of pentobarbitone sodium and severing the jugular vein. This method of slaughter was chosen to minimize the disruption of intestinal mucosa (Badawy, Campbell, Cuthbertson, Fell & Mackie, 1958). In one experiment, to observe the effect of diet and rumen development on the intestinal carbohydrase activities, two calves were weaned when 6 weeks old and thereafter fed on hay and water *ad lib.* plus a normal calf-rearing mixture. The mixture consisted of 47·5% of flaked maize, 24% of bruised oats, 9·5% of extracted decorticated groundnut meal, 6% of dried skim milk, 3% of fish meal and 10% of a mixture of molasses and peat with supplementary vitamins A and D, calcium phosphate, NaCl and trace elements. The calves were killed when about 4 months old and compared with calves of the same age that had been maintained on whole milk. The four adults used in the experiments were killed at the slaughterhouse.

Immediately after slaughter, the abomasum, intestine

and pancreas were removed and chilled with ice, and the pancreas was stored at −20°. The length of the intestine was measured and measured sections were removed from the abomasum, duodenum, jejunum, ileum, caecum and colon. Each section was cut open, washed with water and gently blotted with a piece of cloth. The mucosa was scraped off with a glass slide and weighed. A known quantity of the mucosa (not more than 10g.) was homogenized with 3 vol. of ice-cold 0·15M-NaCl in an MSE homogenizer at top speed for 3min. The volume of the whole homogenate was adjusted to 50ml. with 0·15M-NaCl. The diluted homogenate was centrifuged at 10000*g* for 15min. at 4°, and the supernatant was stored in small quantities at −20°. Under these conditions the carbohydrase activities in the supernatant remained constant for at least 4 weeks. The pancreas was treated in the same way but all enzyme assays were performed immediately after preparation. In some experiments the activities of the supernatant were compared with activities of the whole homogenate to check that the former gave a consistent measure of total activity. Comparisons were made for samples of mucosa from the abomasum, the proximal, middle and distal parts of the small intestine, the caecum, the colon and of the pancreas of a 4-day-old calf, a 25-day-old calf and a 3½-year-old cow. It was found that the activities in the supernatants, expressed as percentages of those in the whole homogenates, were (means ± S.D.) 65·3 ± 4·3, 66·3 ± 2·8, 73·8 ± 7·1, 65·8 ± 3·7, 83·1 ± 6·4 and 95·7 ± 2·4% for intestinal lactase, cellobiase, maltase, trehalase and amylase and for pancreatic amylase respectively. There were no consistent differences in these percentages with the age of the animal or the site in the alimentary tract, though there were small differences between the recoveries of the different enzymes. In subsequent experiments, supernatants were used in preference to whole homogenates because they were more homogeneous and allowed representative sampling more easily.

Determination of enzymic activities. Disaccharidase activities were determined by the method of Dahlqvist (1964). Lactase, cellobiase, and trehalase activities were assayed at pH5·6 and maltase at pH5·8. One unit of disaccharidase activity is that which hydrolyses 1μmole of disaccharide in 60min. at 37°. The relationship between the amount of enzyme present and the amount of disaccharide hydrolysed was studied by varying the amount of homogenate present and the duration of incubation. Results showed that the hydrolysis of each disaccharide (initial concentration 28mM) was directly proportional to the amount of enzyme until at least 10% of the substrate had been hydrolysed. In routine assays the homogenates were diluted to ensure that the total hydrolysis never exceeded 10%.

Dextranase and amylase activities were measured by the method described by Dahlqvist (1961), in which the increase in reducing power of a dextran or starch solution is measured with dinitrosalicylate reagent. The dinitrosalicylate reagent was prepared by dissolving 10g. of 3,5-dinitrosalicylic acid in 200ml. of 2N-NaOH. This solution was diluted with 500ml. of water, 300g. of sodium potassium tartrate was added to it, and, when solution was complete, it was made up to 1l. with water. One unit of dextranase or amylase activity is that which liberates reducing groups corresponding to 1μmole of maltose in 60min. at 37°.

Determination of protein. The protein content of the homogenates was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951). Bovine plasma albumin was used to prepare a standard curve.

RESULTS

Enzymic properties of the small-intestinal disaccharidases

Influence of pH. The pH–activity curves for the disaccharidases studied are shown in Fig. 1. The lactase activity had an optimum pH between 5·4 and 5·7, the cellobiase between 5·5 and 5·8, the maltase between 5·6 and 5·9, and the trehalase between 5·3 and 5·7. Heilskov (1951*a*) found the optimum pH for intestinal lactase of the calf to be about 5·5. The pH optima for the intestinal disaccharidases found in the calf were similar to the values found in other mammalian species (Auricchio *et al.* 1963; Welsh & Walker, 1965).

Effect of substrate concentration. At low substrate concentrations a normal curve was observed for

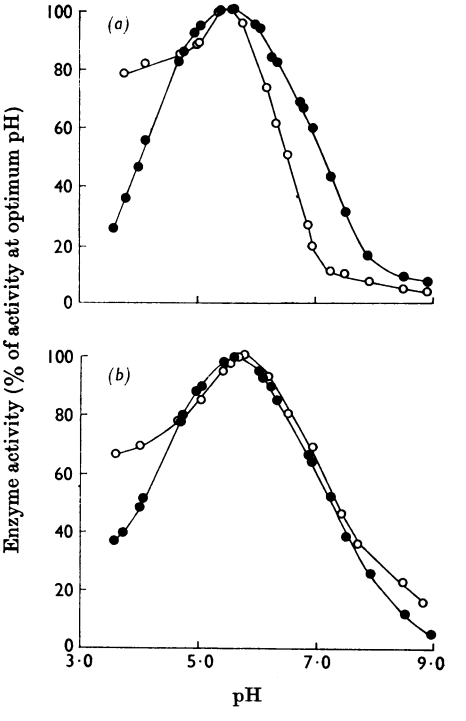

Fig. 1. pH–activity curves for the disaccharidase activities of a homogenate of calf small-intestinal mucosa: (*a*) ○, trehalase; ●, lactase; (*b*) ○, maltase; ●, cellobiase. The enzyme activities are expressed as percentages of the activity at the optimum pH. The buffers used were: pH3·6–5·4, 50mM-sodium acetate–acetic acid; pH5·5–7·0, 50mM-sodium maleate; pH6·2–8·0, 50mM-sodium phosphate; pH7·5–9·0, 25mM-sodium veronal–HCl.

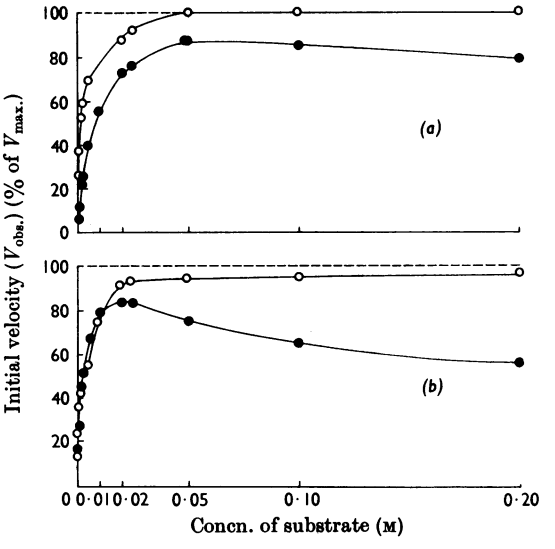

Fig. 2. Disaccharidase activities of calf small-intestinal mucosal homogenates as a function of the substrate concentration: (*a*) ○, trehalase; ●, lactase; (*b*) ○, maltase; ●, cellobiase. Experimental conditions were: temperature 37°; 50mM-sodium maleate buffer, pH 5·6 for lactase, cellobiase and trehalase, and pH 5·8 for maltase. The initial velocities ($V_{obs.}$) are expressed as percentages of the maximal velocities ($V_{max.}$). The latter are shown as broken lines and were calculated graphically as described in the text.

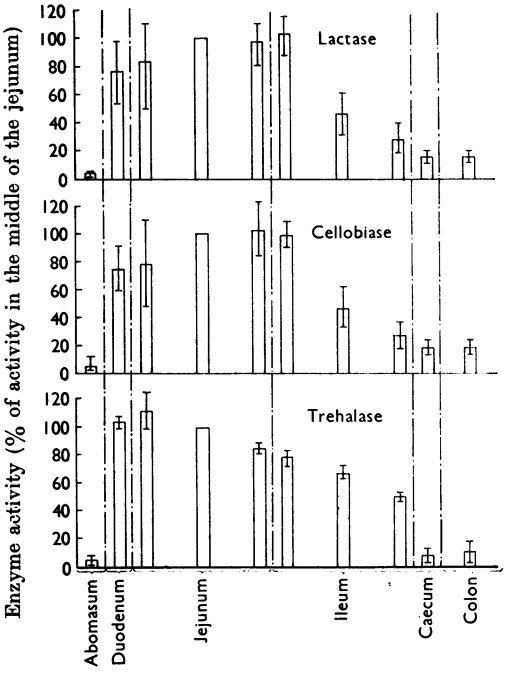

Fig. 3. Disaccharidase activities of homogenates of mucosa from different parts of the digestive tract of the calf. The results are the mean of the activities observed in two 4-day-old calves and one 14-day-old calf, with the S.D. from the mean indicated. The enzyme activities are expressed as percentages of the activity in the homogenates of the mucosa from the middle section of the jejunum. The length of the small intestine was 15·75–19·35m. The duodenum had an approximate length of 0·5–1·0m. and the junction between the jejunum and ileum was taken arbitrarily as halfway along the small intestine.

each disaccharide (Fig. 2). Substrate concentrations between 0·05 and 0·20M, however, caused a marked inhibition of the cellobiase activity; the lactase activity was only slightly inhibited at these substrate concentrations and the maltase and trehalase activities were not affected. Substrate inhibition of intestinal glycosidases has been reported previously (Auricchio *et al.* 1963; Dahlqvist, 1960). In the routine assays 28mM-substrate was employed, and Fig. 2 shows that at this concentration the velocity of the reaction was approx. 80% of the maximum for lactase and cellobiase and 95% for maltase and trehalase.

The K_m value for each of the disaccharidases was calculated graphically by the method described by Lineweaver & Burk (1934) and Dixon (1953). The K_m for lactase was 7·5mM, for cellobiase 2·5mM, for maltase 3·5mM and for trehalase 1·7mM. Heilskov (1951*a*) obtained a value of 5·5mM for the K_m of calf intestinal lactase.

Location of the carbohydrase activities along the gastrointestinal tract

The results in Fig. 3 are the mean of the activities observed in three young calves. In older calves the pattern of distribution remained the same, though there was a change in the enzymic activity with age (see Table 1). A similar distribution of the various carbohydrase activities was obtained when the activities were expressed either as units/mg. of protein or as units/cm. of intestine.

The carbohydrase activities were located mainly in the small intestine. Lactase activity was slightly higher in the middle section of the small intestine than in the proximal section and decreased markedly in the distal section. The cellobiase activity was distributed similarly, suggesting that the two activities might be exerted by the same enzyme. Trehalase activity was highest in the proximal part of the small intestine and decreased distally. Maltase activity was more uniformly distributed along the small intestine, the activity being slightly higher in the distal part than in the proximal part. The amylase and dextranase activities also appeared to be uniformly distributed along the small intestine, though the amylase

Table 1. *Bovine intestinal carbohydrase activities at different ages*

The calves were maintained on whole milk unless otherwise stated. For experimental details see the text.

		Activity (units/mg. of protein)											
Age		4 days	4 days	14 days	25 days	43 days	101 days	101 days*	113 days	114* days	2 years	2½ years	3½ years	6 years
Live wt. (kg.)		34·5	31·5	36·0	41·8	46·7	64·4	91·6	77·5	93·9	—	—	—	—
Small intestine length (m.)		15·75	16·20	19·35	22·50	18·45	20·70	24·30	22·05	25·20	40·50	44·10	38·70	43·65
Region of small intestine														
Lactase	Proximal	4·78	6·26	2·96	1·72	1·88	2·36	3·49	3·14	2·09	0·38	0·37	0·05	0·18
	Middle	8·36	10·90	3·46	1·89	1·95	2·10	2·31	3·02	2·21	0·31	0·13	0·04	0·17
	Distal	4·88	2·70	2·13	0·49	0·39	0·23	0·26	0·50	0·21	0·09	0·05	0·04	0·17
Cellobiase	Proximal	0·62	0·87	0·45	0·26	0·40	0·33	0·55	0·53	0·31	0·06	0·10	0·00	0·03
	Middle	1·26	1·56	0·46	0·28	0·26	0·31	0·28	0·55	0·32	0·06	0·07	0·00	0·04
	Distal	0·67	0·38	0·22	0·07	0·08	0·05	0·03	0·10	0·04	0·04	0·05	0·00	0·05
Amylase	Proximal	0·42	0·19	0·67	0·29	0·59	2·38	0·93	0·28	1·01	0·72	3·17	5·07	1·11
	Middle	0·44	0·14	0·91	0·30	0·70	1·06	1·35	1·81	0·86	1·45	2·60	2·94	3·02
	Distal	0·45	0·09	2·05	0·46	0·67	1·50	0·95	1·73	1·04	1·82	3·17	0·43	0·53

* Weaned when 41 days old and thereafter fed on hay and water *ad lib.* plus a normal calf-rearing mixture. The composition of the calf-rearing mixture is given in the Materials and Methods section.

Table 2. *Changes in bovine pancreatic amylase and maltase activities with age*

Conditions were as described in Table 1 and the text.

Age	Wt. of pancreas (g.)	Activity (units/g. of pancreas) Maltase	Amylase
4 days	30·10	8·75	7560
4	41·0	13·83	4985
14	28·3	20·00	7980
25	35·5	15·70	14550
43	53·2	19·35	13420
101	36·7	19·62	97100
101*	91·6	12·12	55450
113	76·6	11·60	50830
114*	104·1	19·60	122050
2 years	369·4	20·85	137500
2½	315·5	15·25	132500
3½	310·5	19·00	280800
6	363·3	15·28	134500

* Weaned when 41 days old.

activity tended to vary irregularly in the individual calves.

Although most of the carbohydrase activities were present in the large intestine, they were present only in small amounts compared with the activities in the small intestine. Maltase was the only enzyme that was present in similar activities in the large and the small intestine. Sucrase and palatinase activities were not detected in any part of the gastrointestinal tract.

Carbohydrase activities in the small intestine of calves of different ages

Lactase and cellobiase activities were highest in the 4-day-old calf and decreased with age up to 43 days but changed little between 43 and 114 days (Table 1). A further decrease in activity was observed between the 114-day-old calf and the adults. The decrease in the lactase and cellobiase activities was more marked in the distal section of the small intestine than in the proximal and middle sections. The maltase, trehalase and dextranase activities were very much lower than the lactase activity and did not change with age. Mean activities $\pm$S.D. were: maltase $0{\cdot}29 \pm 0{\cdot}10$ unit/mg. of protein; trehalase $0{\cdot}13 \pm 0{\cdot}06$ unit/mg. of protein; dextranase $0{\cdot}10 \pm 0{\cdot}03$ unit/mg. of protein.

The intestinal amylase activity increased slightly with age, maximal activity being attained by 101 days and remaining relatively constant thereafter. Sucrase and palatinase activities were not detected in any of the calves or in the adults. No marked differences were observed in the carbohydrase activities of the intestinal mucosa of 101-day-old

and 114-day-old calves that had been fed solely on milk and calves of the same age that had been given a concentrate–hay diet from 6 weeks of age.

Carbohydrase activities of the pancreas

Homogenates of the pancreas exhibited only two carbohydrase activities: a very active amylase and a weak maltase activity. The maltase activity (expressed as units/g. of pancreas) did not change significantly with age, the activity in the 4-day-old calf being similar to the activity in the adult (Table 2). The amylase activity, on the other hand, increased with age, and even in the 114-day-old calf it had not reached that observed in the adult.

DISCUSSION

On the assumption that the disaccharidase activities in intestinal mucosal homogenates reflect the ability of an animal to utilize various carbohydrates, the present results show that the young calf is readily able to utilize lactose but possesses limited ability to utilize cellobiose, maltose and trehalose, and is unable to utilize sucrose. These results are consistent therefore with the results obtained in blood-sugar studies (Velu *et al.* 1960; Okamoto, Thomas & Johnson, 1959), in which dietary lactose caused a marked increase in the blood-sugar concentration of the young calf, whereas maltose and sucrose caused very little or no increase.

The finding of high intestinal lactase activity in the young calf with a subsequent decrease in activity with age is in accordance with observations in other mammals. In the rat (Alvarez & Sas, 1961), rabbit (Doell & Kretchmer, 1962), dog (Welsh & Walker, 1965) and pig (Walker, 1959*a*) the lactase activity is highest at birth and decreases during the weaning period. The stimulus for this decrease in lactase activity is unknown (Herzenberg & Herzenberg, 1959). The fact that there were no marked differences in the disaccharidase activities in 4-month-old calves fed solely on milk and calves of the same age that had been weaned when 6 weeks old suggests that dietary lactose is not important in the regulation of intestinal lactase. Alvarez & Sas (1961) and Heilskov (1951*b*) found that the continued feeding with lactose was ineffective in maintaining high lactase activity in the rat and rabbit. Fischer (1957) reported larger amounts of lactase activity in lactose-fed rats due to an increase in the weight of the intestinal mucosa; the specific activity, however, did not increase.

Unlike many species (e.g. see Rubino *et al.* 1964) the calf showed no increase in intestinal maltase and trehalase activities, and even in the adult cow the activities of these enzymes were similar to those in the young calf. Huber, Jacobson, Allen & Hartman (1961*c*) also found that the intestinal maltase activity of the calf was independent of age. Dollar & Porter (1959), on the other hand, reported greater maltase activity in 64-day-old calves than in 17-day-old calves. The finding of no increase in maltase activity with age is not consistent with the studies of Larsen *et al.* (1956) and Huber *et al.* (1961*b*), in which a significant utilization of maltose was observed in older calves. However, this increase in maltose utilization in older calves may be due to (i) an increase in the total intestinal maltase activity due to an increase in the size of the intestine (the results in Table 1 are recorded per mg. of protein), or (ii) an increase in the amount of maltase activity secreted into the intestinal lumen in the pancreatic juice. McCormick & Stewart (1967) found that there is an increase with age in the volume of pancreatic juice secreted by the calf.

The absence of sucrase activity from the intestine of the calf has been reported by Dollar & Porter (1957, 1959) and by Huber *et al.* (1961*c*). Our studies confirm their findings and also show that the adult cow possesses no intestinal sucrase activity. Fructose, one of the hydrolysis products of sucrose, is not utilized by the calf and when present in the diet causes severe diarrhoea (Velu *et al.* 1960). An absence of intestinal sucrase activity has also been reported in the sheep (Manunta & Nuvole, 1966; Walker, 1959*b*).

As the disaccharidases exert their physiological activity intracellularly (Chain, Mansford & Pocchiari, 1960; Miller & Crane, 1961), the location of optimum activity of the various disaccharidases along the intestine may reflect the site of absorption of the disaccharides. In the calf, the pattern of distribution of the disaccharidases along the small intestine is similar to that found in the pig (Dahlqvist, 1961). A non-uniform distribution of these activities has been observed in the small intestine of other animals (Malhotra & Philip, 1964, 1965).

In addition to the disaccharidases, homogenates of the intestinal mucosa of the calf possessed amylase and dextranase activity. The possibility that the amylase activity may be due to the contamination of the mucosa with intestinal contents containing pancreatic amylase cannot be ruled out, though it seems unlikely from the uniform distribution of amylase activity along the small intestine.

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