

Digestion and absorption of starch, maltose and lactose by the preruminant calf

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1. Preruminant calves, some of which were equipped with re-entrant cannulas in the small intestine, were given liquid feeds in which various starches, maltose or lactose formed the sole source of carbohydrate.
2. The sugars left the abomasum at the same rate as a water-soluble marker but the starches were greatly delayed. Little starch left the abomasum until about 5 h after feeding. Examination of abomasal contents 0.5 h after feeding showed the starch to be associated with the casein clot.
3. Amounts of starch, maltose and lactose removed during the passage of digesta through the small intestine were about 60, 43 and 97 % of intake respectively, with no marked difference with age between about 7 and 16 weeks.
4. No change in blood glucose was apparent in calves given starch-containing feeds. The possible effect of the retention of dietary starch in the abomasum in minimizing blood glucose responses is discussed.

Digestibility trials have indicated that dietary starch is poorly utilized by calves up to 4–6 weeks of age (Shaw, Woodward & Norton, 1918; Huber, Jacobson, McGilliard, Morrill & Allen, 1961), but the extent of its digestion, posterior to the rumen, in older calves is not well defined. Larsen, Stoddard, Jacobson & Allen (1956) reported that even in 9-month-old calves most of the starch introduced directly into the abomasal region of the stomach appeared undigested in the faeces. Other workers, on the other hand, have reported fairly high apparent starch digestibilities (about 60–80 %) in the whole intestine of older calves, although fermentation in the large intestine may contribute to this (Shaw *et al.* 1918; Noller, Ward, McGilliard, Huffman & Duncan, 1956; Huber, Jacobson, McGilliard, Morrill & Allen, 1961; Natrajan, Polan, Chandler, Jahn & Huber, 1972).

The inclusion of starch as a major energy source in milk-replacer diets for young calves has resulted in poor growth and in digestive disturbances (Flipse, Huffman, Duncan & Webster, 1950; Noller, Ward & Huffman, 1956), although the results of a more recent investigation by Mathieu & Thivend (1968) showed that the daily weight gain of calves up to 13 weeks of age given small amounts of raw starch was equal to or greater than that of similar animals given amounts of whole milk of equal energy value. There is also some indication that the growth of calves on high-starch diets improves after the first few weeks of life (Noller, Ward & Huffman, 1956).

The lack of response in blood sugar concentration when starch has been fed to the calf has been interpreted as showing poor utilization of this carbohydrate (Flipse *et al.* 1950; Larsen *et al.* 1956; Dollar & Porter, 1957, 1959; Okamoto, Thomas & Johnson, 1959; Huber, Jacobson, McGilliard & Allen, 1961). This lack of response has

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been observed also in 2-year-old animals in which the diets were introduced directly into the abomasal region of the stomach (Huber, Jacobson, McGilliard & Allen, 1961).

The extent to which the older preruminant calf is able to derive energy from dietary starch appears, therefore, to be still uncertain and, in the present work on carbohydrate utilization by such calves, particular emphasis has been given to this problem. An attempt has been made to account for some of the apparent discrepancies reported in the literature.

EXPERIMENTAL

Animals and feeding

Castrated Friesian bull calves, aged between 4 and 20 weeks, were used in all the experiments in which intestinal cannulas were employed. Three calves (1A, 2A and 3A) were fitted with re-entrant cannulas immediately below the pyloric sphincter; three other calves (1C, 2C and 3C) were fitted with re-entrant cannulas in the most distal part of the ileum. The calves were operated on between 2 and 6 weeks of age and allowed to recover for at least 2 weeks before being used in experiments. Guernsey bull calves (1B, 2B, 3B, 1D, 2D, 3D, 4D) were used in all other experiments.

All calves were reared on whole-milk diets given in amounts sufficient to maintain a body-weight increase of 0.25 kg/d (Roy, Shillam, Hawkins & Lang, 1958). Milk given to calves over 3 weeks of age was supplemented with iron, manganese, copper and magnesium, vitamins A and E and cholecalciferol. For experiments, calves were usually given synthetic milk feeds containing lactose, maltose or starch as the carbohydrate source. The starches used were uncooked products from potato, rice, maize and wheat or a commercial product known as 'soluble' starch (BDH Chemicals Ltd, Poole, Dorset). 'Soluble' starch is prepared by the partial acid-hydrolysis of potato starch. An experimental feed was prepared by dissolving or dispersing 200 g of the carbohydrate under investigation in 1.9 kg water to which 41 ml of a sodium citrate-citric acid solution (solution A, see below) had been added. Calcium caseinate (110 g) was then dispersed in this solution, and an emulsion of 35 g margarine plus 1.7 g Lubrol W (Imperial Chemical Industries Ltd, Blackley, Manchester) in 150 ml water was added. Three mineral solutions were prepared containing, respectively, in 1 l: (A) 22 g NaOH and 20 g citric acid; (B) 11 g Na_2HPO_4 , 70 g K_2HPO_4 and 15 g KH_2PO_4 ; (C) 142 g $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ and 50 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. Portions of solution A (62 ml), solution B (104 ml) and solution C (51 ml) were mixed and added to the feed and the whole mixture was homogenized for 5 min in a Silverson homogenizer (Model AX; Silverson Machines, London SE1).

Analytical methods

In some experiments phenol red was used as a marker and was therefore present in samples of ileal digesta. It was removed by shaking 10 ml of the sample with about 0.25 g of Amberlite IRA-400 ion-exchange resin (chloride form) for approximately 15 min.

Starch. Standard solutions containing 0–100 g of 'soluble' starch/l were prepared and 1 ml samples were added to 25 ml 2 M-HCl and refluxed for 90 min in a boiling

water-bath. After cooling, the pH was adjusted to between 6.8 and 7.2 with 2 M-NaOH and the solution diluted to 100 ml. Samples (1 ml) of digesta were treated in the same way, the final solution being suitably diluted to give concentrations corresponding to the standards. Glucose was determined in the hydrolysates by the glucose oxidase method of Dahlqvist (1961). For six starch solutions containing 20–100 g/l, the mean recovery of glucose (\pm SEM) was $96.5 \pm 3.2\%$.

Monosaccharides and disaccharides. Before analysis, all samples (i.e. blood, duodenal digesta or decolorized ileal digesta) were deproteinized. A sample (1 ml) was diluted with 3 ml distilled water and 0.5 ml of 0.15 M-barium hydroxide and 0.5 ml of zinc sulphate (50 g/l) were added and the mixture was filtered. Glucose was estimated in the filtrate by the tris-glucose oxidase method (Dahlqvist, 1961). Lactose and maltose were estimated from the total reducing sugar content of the filtrate as measured by an automated copper-neocuproine method (Technicon Bulletin, Technicon Instruments Company, Ltd, Basingstoke, Hants) corrected, if necessary, for glucose content.

Polyethylene glycol. Polyethylene glycol (PEG) in digesta samples was measured by the method of Smith (1958, 1962), but with a 20 min time for development of turbidity.

¹⁴C-labelled PEG. Digesta samples were homogenized for 5 min at top speed in an Ato-Mix blender (Measuring and Scientific Equipment Ltd, London). After centrifugation for 45 min at 1000 g, the supernatant fraction was decanted off; 1 ml of it was shaken with 1 ml 1 M-hyamine hydroxide in methanol (Nuclear Enterprises Ltd, Edinburgh). This was then mixed with 10 ml phosphor reagent (0.3 g 1,4-bis(5-phenyl-2-oxazolyl)-benzene, 4 g diphenyl oxazole, 80 g naphthalene, 430 ml distilled anisole, 560 ml distilled methoxyethanol). Concentrated HCl (0.1 ml) was then added and radioactivity was measured by liquid β -scintillation counting in a Packard Tri-Carb Liquid Scintillation Spectrophotometer, Model 3003. ¹⁴C-labelled toluene was used as an internal standard checked against n-[1-¹⁴C]hexadecane reference material (supplied by the Radiochemical Centre, Amersham).

Procedure

Passage of carbohydrates from the abomasum. Experimental feeds containing 200 g carbohydrate and 25 μ Ci [¹⁴C]PEG were given to the calves fitted with duodenal cannulas (1 A, 2 A and 3 A) and digesta samples from the efferent cannulas were collected in approximately 50 g fractions over periods of 6–9 h. In each experiment, fractions were automatically recorded and most then immediately returned to the afferent cannula. At approximately hourly intervals fractions were removed for analysis and replaced by equal volumes of a solution containing 8.06 g NaCl, 50 g glucose and 1.75 ml concentrated HCl per l distilled water (pH approx. 1.6). Infusion of this solution into the duodenum of a calf has been shown (unpublished results) to have a similar effect on the rate of abomasal emptying as the digesta leaving the abomasum after a feed of milk.

Recovery of material at the terminal ileum. The calves (1 C, 2 C and 3 C) fitted with re-entrant ileal cannulas were given feeds of the experimental diet containing 200 g of an appropriate carbohydrate, 5 g PEG and 0.1 g phenol red. Phenol red was included in these diets to indicate the arrival of feed residues at the terminal ileum (Smith, 1964).

Once phenol red had appeared (approximately 3 h after feeding) ileal effluent was collected. After a feed containing a disaccharide, collection continued for three consecutive 1 h periods; after a feed containing starch, collection continued for a total period of about 21 h (two initial 1.5 h periods and one 18 h period). No further feed was given during the collection periods but 2.2 kg water were given 8 h after a starch feed.

Slaughter experiments. Three calves (1 B, 2 B and 3 B) aged 9 weeks were given the experimental diet containing 200 g wheat starch and 5 g PEG. Thirty minutes after feeding, each calf was slaughtered by an intravenous injection of an overdose of veterinary Nembutal (Abbott Laboratories Ltd, Queenborough, Kent). The abomasum was exposed and tied off. The abomasal contents were collected and filtered through fine gauze. The volume of the filtrate was then measured and a sample taken for analysis. The clot fraction retained in the gauze was homogenized in distilled water and made up to the same volume as that of the supernatant fraction. A sample was removed for analysis.

Blood-sugar experiments. Four calves (1 D, 2 D, 3 D, 4 D), aged 0.5–5 weeks, were given a feed of whole milk at 17.00 hours on the day preceding the experiment. After an overnight fast, they were given feeds of the experimental diet containing 200 g 'soluble' starch or a control feed containing no carbohydrate. Samples of blood were taken through an indwelling 150 mm nylon cannula (Portex nylon tubing – size 3 V, i.d. 1.0 mm; Portex Ltd, Hythe, Kent) inserted into the jugular vein at least 30 min before feeding. Blood samples were taken immediately before feeding and at hourly intervals over the 12 h after feeding.

RESULTS

Passage of carbohydrates from the abomasum

Flows of lactose, maltose or various starches from the abomasum relative to [¹⁴C]PEG were determined after calves had been given appropriate diets. The calves were 8–18 weeks of age and, within this range, no differences with age were apparent. Typical detailed results for lactose after calf 1 A had been given the experimental diet containing this sugar are shown in Fig. 1. Replicate experiments with this and another calf (2 A) (seven experiments in all) gave closely similar results. Similar results were also obtained for lactose after a feed of cow's milk had been given (seven experiments) and for maltose when this sugar replaced lactose in the experimental diet (seven experiments). For both these sugars, ratios of carbohydrate to PEG in samples of digesta taken at different times after feeding remained virtually identical to the corresponding ratio in the diet. This is illustrated by the results in Table 1. A very different pattern of carbohydrate appearance was, however, apparent in six similar experiments in which calves (1 A and 2 A) were given the experimental diet containing 'soluble' starch. Digesta samples taken in the first few hours after feeding showed starch:PEG ratios much lower than the ratio in the diet, whereas samples taken at about 6 h after feeding showed high ratios. Mean values are given in Table 1 and typical patterns of appearance of starch and PEG in duodenal contents are illustrated in Fig. 2. Similar results (Table 1) were obtained with another calf (3 A) in whose diet 'soluble' starch was replaced by

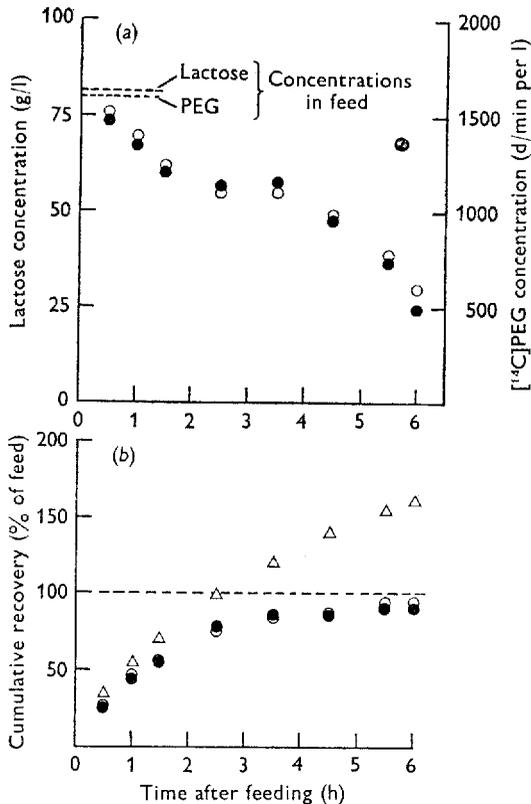


Fig. 1

Fig. 1. (a) Concentrations of lactose (○) and polyethylene glycol (PEG) (●) in samples of abomasal effluent taken from a preruminant calf, 1 A, at different times after ingestion of a lactose-containing experimental feed (see p. 228). (b) Cumulative recovery of water (△), lactose (○) and PEG (●) in the same experiment.

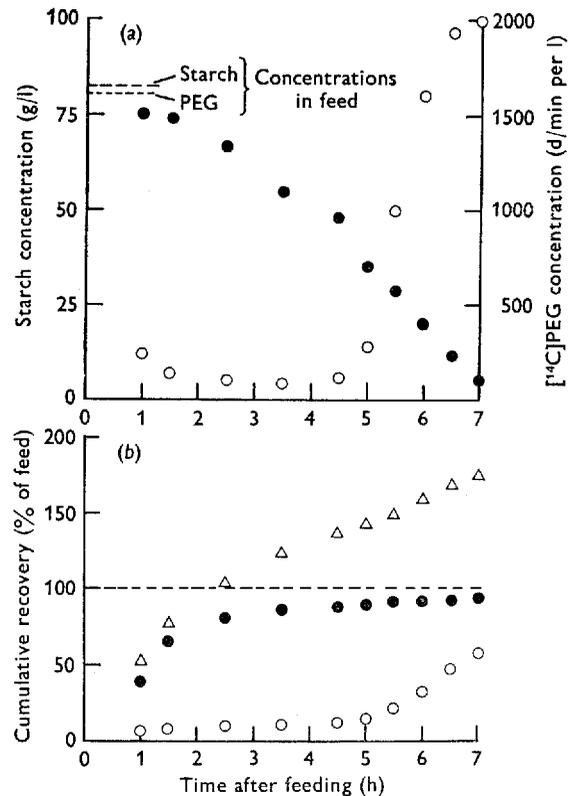


Fig. 2

Fig. 2. (a) Concentrations of starch (○) and polyethylene glycol (PEG) (●) in samples of abomasal effluent taken from a preruminant calf, 1 A, at different times after ingestion of an experimental feed containing 'soluble' starch (see p. 228). (b) Cumulative recovery of water (△), starch (○) and PEG (●) in the same experiment.

various raw starches. In all the experiments in which starches were included in the diet the average rates of passage of digesta (approximately 715 g in the first 4 h) and rates of recovery of PEG (approximately 65% after 2 h and approximately 90% after 6 h) at the duodenum were closely similar to those shown in the maltose or lactose experiments (corresponding values 720 g/h, 70% and 90% respectively).

These results showed that starch was held back in the abomasum, relative to water-soluble constituents, for a period after feeding. This retention was investigated more directly by examining the abomasal contents of three calves (1 B, 2 B and 3 B) slaughtered 30 min after they had been given the experimental diet containing wheat starch and PEG. The amounts of PEG and starch found in the supernatant and clot fractions were 4.46 ± 0.19 g (mean \pm SEM for three calves) and 0.21 ± 0.06 g respectively for PEG, and 24.6 ± 5.1 g and 152.2 ± 10.1 g respectively for starch. This showed that starch was largely associated with the casein clot in the abomasum at that time.

Table 1. Ratios of carbohydrate to polyethylene glycol (PEG) in samples of abomasal effluent of preruminant calves taken 1 and 6 h after ingesting feeds containing different carbohydrates, compared with the corresponding ratios in the diets. Except for one group of experiments with cow's milk all feeds were prepared as described on p. 228

(Mean values with their standard errors)

Dietary carbohydrate	Calves	No. of expts	Diet	Ratio, carbohydrate:PEG	
				Digesta	
				1 h after feeding	6 h after feeding
Lactose (in cow's milk)	1 A and 2 A	7	5.28 ± 0.09	5.32 ± 0.14	5.36 ± 0.08
Lactose (in synthetic diet)	1 A and 2 A	7	5.34 ± 0.10	5.38 ± 0.16	5.36 ± 0.21
Maltose	1 A and 2 A	7	5.30 ± 0.09	5.35 ± 0.11	5.40 ± 0.10
Soluble starch	1 A and 2 A	6	5.29 ± 0.08	0.99 ± 0.35	22.28 ± 8.23
Rice starch	3 A	3	5.30 ± 0.12	1.58 ± 0.21	22.01 ± 5.21
Maize starch	3 A	3	5.26 ± 0.08	0.18 ± 0.03	23.88 ± 6.32
Wheat starch	3 A	4	5.34 ± 0.09	0.17 ± 0.02	33.82 ± 9.48
Potato starch	3 A	3	5.28 ± 0.10	0.16 ± 0.04	47.79 ± 6.14

Table 2. Disappearance of dietary carbohydrates from the small intestine up to the ileum of calves 6-9, 9-14 and 15-16 weeks old

(Mean values with their standard errors for three calves, calculated from the difference between the carbohydrate:polyethylene glycol ratio in the diet and in ileal digesta)

Dietary carbohydrate	Disappearance (% of dietary carbohydrate)		
	6-9 weeks	9-14 weeks	15-16 weeks
Lactose	97.8 ± 1.0	98.8 ± 0.5	95.4 ± 1.5
Maltose	45.7 ± 4.3	39.7 ± 6.4	43.5 ± 7.4
Soluble starch	60.6 ± 5.7	62.7 ± 5.1	56.2 ± 4.2

Digestion and absorption of carbohydrates in the small intestine

Net removals of lactose and maltose (and their constituent monosaccharides) up to the distal ileum were determined in three calves (1 C, 2 C, 3 C) by giving suitable experimental diets, collecting digesta samples at the ileum for periods of 3 h after the first arrival of food residues and comparing recoveries of sugar with those of PEG. Results, given in Table 2, showed lactose to be almost completely removed (the very small amount of surviving sugar consisted mainly of galactose (cf. Coombe & Smith, 1973)). Maltose was removed much less efficiently and, usually, more than half was recovered at the ileum (virtually no free glucose was present). This technique for estimating disaccharide disappearance up to the ileum could not be used for comparable estimations of starch disappearance because of the hold-up of starch in the abomasum in association with the casein clot. It is known (unpublished observations), however, that very little of a casein clot survives after 24 h in the abomasum. Results for starch disappearance up to the ileum (Table 2) are based, therefore, upon collections for 24 h after an experimental feed, during which time the calf received only water. Starch

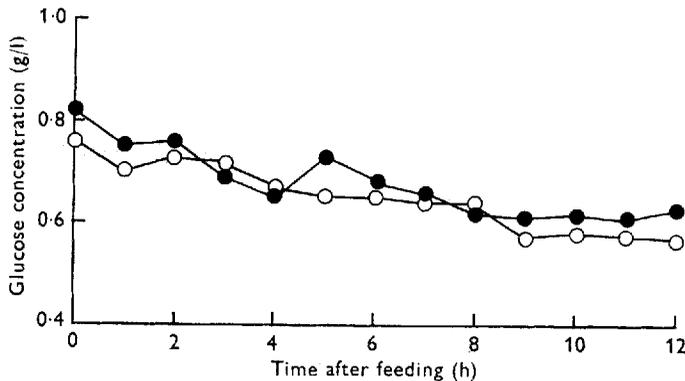


Fig. 3. Concentrations of glucose in the jugular blood of a calf aged 4–5 weeks after the ingestion of experimental feeds (see p. 228) containing 'soluble' starch (○) or no added carbohydrate (●).

disappearance appeared to be somewhat greater than that of maltose although it was far from complete. No free maltose or glucose was detected in ileal contents after a starch feed. The calves used were aged 6–16 weeks and, within this range, no differences due to age were apparent (Table 2).

Blood-sugar responses to the ingestion of starch

Changes in blood sugar concentration soon after a feed have frequently been used to assess the availability of carbohydrate in that feed. The hold-up of starch in the abomasum is, however, likely to invalidate the application of such a method to diets containing this carbohydrate and may, in part, account for failures to detect changes in blood glucose concentration after starch-containing feeds have been given (e.g. Larsen *et al.* 1956; Okamoto *et al.* 1959). The possibility that a delayed rise in concentration of blood glucose may occur after such diets have been given was examined in experiments with four calves (1 D, 2 D, 3 D, 4 D). Two experiments were made with each calf, the first at about 1 week of age, the second at 4–5 weeks. Each experiment consisted of two 12 h collections of blood samples, one after a morning experimental feed containing 'soluble' starch, and the other after a feed that was similar but contained no carbohydrate. No further food was given during the collection periods. There were no marked differences between the different experiments and none showed any evidence of a delayed rise in concentration of blood glucose. A typical example is illustrated in Fig. 3.

DISCUSSION

It is well established that the very young calf can make little use of dietary starch; the amounts of pancreatic amylase secreted by a calf of about 1 week of age are very low (Ternouth, Siddons & Toothill, 1971), and young calves given synthetic diets with starch as the main carbohydrate do not thrive (Flipse *et al.* 1950; Dollar & Porter, 1957; Natrajan *et al.* 1972). The amounts of pancreatic amylase increase, however, in the first few weeks of a calf's life (Siddons, 1968; Morrill, Stewart, McCormick & Fryer,

1970; Ternouth *et al.* 1971). The small-intestinal mucosa possesses both maltase and isomaltase activities (Siddons, 1968; Coombe & Siddons, 1973), although these are present in much smaller amounts than lactase and are low compared with the amounts found in the small-intestinal mucosa of some other animals, e.g. the pig (Dahlqvist, 1960), rat (Doell & Kretchmer, 1962), and man (Auricchio, Rubino, Tosi, Semenza, Landolt, Kistler & Prader, 1963). It would be expected from these facts that the older calf would have a limited ability to digest and use starch. The available evidence suggests that this is so but the extent of the limitation is not well defined.

One possible reason for the lack of agreement on this point is provided by our finding that starch is retained in the abomasum apparently in association with the casein clot (Fig. 2). Some workers (Dollar & Porter, 1957, 1959; Okamoto *et al.* 1959) have reported little or no response in blood glucose concentration after giving starch-containing diets to calves. These findings in older calves may have been partly due to a retention of starch in the abomasum. It might be expected that with such a retention a delayed increase in blood glucose might occur. We failed to detect an increase in calves up to 5 weeks of age (Fig. 3). This may have been because their pancreatic amylase secretion was not fully developed, and further experiments with older calves are desirable. A small rise in concentration of blood glucose between about 5 and 8 h after calves of 12 weeks and older had been given a starch-containing feed has been reported by Natrajan *et al.* (1972). However, failure to detect more than a small rise in blood glucose concentration is not necessarily indicative of negligible glucose absorption; with retardation of starch movement into the duodenum, it is possible that absorption occurs over a long time, so that glucose is metabolized in either the mucosa of the small intestine or the liver as rapidly as it is absorbed. This possibility is consistent with the results of Gropp (1971) obtained with a calf given a starch diet for 10 weeks. The calf showed only small changes in systemic concentration of blood glucose after feeding but marked increases in portal concentrations of blood glucose both soon after feeding and about 6 h later. This latter increase corresponded approximately to the time of peak movement of starch into the duodenum observed in our calves.

Retention of starch in the abomasum could also affect the assessment of its utilization in experiments in which short-term measurements of its disappearance from intestinal digesta were made. By making 24 h collections of ileal digesta we have avoided most of the potential errors associated with uneven flow of starch from the abomasum, although the possibility of a little starch remaining in the abomasum cannot be entirely discounted. Such a retention could lead to a small over-estimate of digestibility and may perhaps account in part for the fact that starch digestibility appeared to be somewhat greater than maltose digestibility (Table 2). Nevertheless, it seems likely that for these older calves, starch digestion in the small intestine was at least as efficient as maltose digestion. This suggested that amylase activity did not limit starch digestion. On the other hand, if maltase activity were limiting, accumulation of maltose in the small intestine might be expected, and this did not occur. Our findings suggest therefore that amylase and maltase (and isomaltase) activities were approximately balanced. This is a tentative conclusion which requires further investigation. It is different from that which would be reached from a study of changes in blood glucose if the effects of

starch retention were ignored, since dietary maltose causes a greater increase in blood glucose soon after feeding than does dietary starch (Huber, Jacobson, McGilliard, Morrill & Allen, 1961; Siddons, Smith, Henschel, Hill & Porter, 1969; Gropp, 1971). These considerations apply only to the older calves which we examined; the much lower pancreatic amylase secretion shown by very young calves (cf. Ternouth *et al.* 1971) suggests that, for these animals, amylase is probably limiting.

The extent of starch digestion in the small intestine which we have found (about 50–70%) is somewhat less than values for disappearance in the whole intestine of calves of similar age (70–80%) found by some other groups of workers (Huber, Jacobson, McGilliard, Morrill & Allen, 1961; Natrajan *et al.* 1972) probably because fermentation in the large intestine led to some disappearance of starch. Our results agree approximately with values for the digestibility of α -linked glucose polymers in the small intestine of ruminating calves (about 65%) found by McAllan & Smith (1972). It is not at present known whether amylase activity, maltase (or isomaltase or both) activity or both limit the digestion of starch-like material in the small intestine of the ruminant.

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