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Effect of guar gum on nitrogen secretion into isolated loops of jejunum in conscious growing pigs

BY A. G. LOW AND ANNA L. RAINBIRD

National Institute for Research in Dairying, Shinfield, Reading, Berks RG2 9AT

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1. Four pigs, initially of 30 kg live weight, were surgically prepared with two re-entrant cannulas in the jejunum, 1·0 m apart. This allowed an isolated loop to be formed through which Ringer solutions were continuously perfused for 6·5-h periods per day.

2. The effects of two Ringer solutions of contrasting composition on nitrogen secretion were measured in a preliminary study.

3. The effects of guar gum (6·7 g/l) on N secretion during perfusions of solutions of glucose or of maltose (20 g/l) were measured.

4. N secretion did not differ significantly between the two Ringer solutions.

5. Addition of guar gum to the solution of glucose increased mean N secretion from 69·2 to 133·9 mg/m per 2 h; the corresponding values for the maltose solution were 75·5 and 120·2 mg/m per 2 h. In both cases the differences were significant ($P < 0.001$). Differences between N secretion into glucose and maltose solutions were not significant, either without or with guar gum.

6. Guar gum addition had a greater effect on N secretion in hours 5 and 6 than in hours 1 and 2 of perfusion.

7. It was calculated that guar gum would have increased N secretion by the mucosa of the whole small intestine from approximately 15 to 27 g/d under the conditions of the study, assuming uniform secretion throughout the organ. These results suggest that certain types of dietary fibre may be important determinants of N secretion by the mucosa of the small intestine. They also suggest that changes in N secretion of this magnitude are of importance in N metabolism because the overall rate of protein synthesis in these pigs was probably about 100 g/d (expressed as N), using values from the literature.

Studies in recent years have shown that the digestive tract of growing pigs is a site of rapid protein synthesis and turnover (for example, Simon *et al.* 1983). It has also been shown by many authors that large amounts of nitrogenous compounds are secreted into the gut lumen of pigs during digestion (Juste, 1982), and it is thus likely that these are closely related processes. The exact amounts of nitrogen secreted into the gut are not accurately known but it is clear that changes in the nutrition of pigs can cause large alterations in these quantities. A general scheme of the approximate daily inputs of N into the gut of pigs has been constructed by Low (1982), who also reviewed some of the nutritional factors which may influence this process.

The mucosa of the small intestine is a particularly important secretory tissue, since more N is secreted by it than by the stomach or the liver (as bile) or the pancreas. Using 50–60 kg pigs, Buraczewska (1979) measured the amount of N secreted into isolated loops of various regions of the small intestine which were perfused with solutions based on Krebs–Ringer solution. It was calculated from these studies that about 15 g N were secreted per day by the whole small intestine. The mean daily amount of N secreted into isotonic Krebs–Ringer solution during perfusion of the jejunum was 0·97 g N/m.

In view of the fact that the amounts of N secreted into the gut may be equivalent to as much as 20–25% of daily protein synthesis (Buraczewska, 1979; Reeds *et al.* 1980; Simon *et al.* 1983) it was of interest to investigate how nutritional factors may influence this process of considerable metabolic importance. Guar gum (a galactomannan which increases the viscosity of solutions) was chosen for use in the present study because it is known to affect gut function by reducing the rate of glucose absorption in man (Jenkins *et al.* 1978); this is of considerable importance in the nutritional management of diabetes. The physiological

basis of this effect is not understood fully and so we have recently been using pigs as models for man to investigate this further, preliminary studies having indicated their suitability (Leeds *et al.* 1980). We have shown in the previous paper (Rainbird *et al.* 1984) that part of the effect of guar gum appears to be to reduce the rate of glucose absorption in the jejunum. The effect was similar irrespective of whether the absorbed glucose was from solutions of glucose or maltose (indicating that the absorption process was not limited by maltase (*EC* 3.2.1.20)). In the previous paper we compared the effects of Krebs–Ringer solution and a new Ringer solution with a composition resembling more closely the ionic content of digesta found in the jejunum of pigs given similar diets (Partridge, 1978). Although there were no significant differences in glucose absorption from these two Ringer solutions, their effects on N secretion were also of interest and are reported here.

EXPERIMENTAL METHODS

Animals and surgery

Four male Large White × Landrace pigs of approximately 30 kg live weight were surgically prepared with two re-entrant cannulas 1·0 m apart in the jejunum. Full details are given by Rainbird *et al.* (1984).

Diet and feeding

The composition of the diet was (g/kg air-dry diet): maize starch 560·9, soya-bean meal 300·0, Solkafloc 60·0, maize oil 30·0, minerals and vitamins 49·1 (see Rainbird *et al.* 1984, Table 1). The pigs were weighed weekly and fed at a level of 42·5 g/kg body-weight per d in two equal meals at 09.00 and 21.00 hours. The diet was mixed with water (1:2·5, w/v) immediately before feeding.

Perfusion technique

Two Ringer solutions were used (see Rainbird *et al.* 1984, Table 2). The first was a classical Krebs–Ringer and the second was based on this solution, the ionic composition of which resembles that of blood serum. However, Partridge (1978) showed that the ionic composition of digesta from the jejunum of pigs fed on diets similar to those used in the present study differed markedly from that used in Krebs–Ringer, with respect to sodium and potassium. The second solution used here (called new Ringer) was therefore prepared to resemble as closely as possible the ionic composition of digesta in the jejunum.

Perfusions lasted 6·5 h/d. Before the morning feed the exit part of the proximal cannula was connected to the entry part of the distal cannula; the entry part of the proximal cannula was connected to a peristaltic pump and the exit part of the distal cannula drained through a tube into a bottle surrounded by ice. The perfused solutions were gassed with oxygen–carbon dioxide (95:5, v/v), kept at 40° and pumped into the isolated loop at 8 ml/min (the mean rate of digesta flow in this region in the same pigs during preliminary measurements before the perfusions). The loop was initially washed out with 50 ml of the solution to be used during the rest of the day, followed by 30 min of perfusion; this perfusate was then discarded. During the following 6 h the volume of the perfusate collected was measured (at 2-h intervals), sampled and the residue discarded.

The perfused solutions were marked with ^{51}Cr-EDTA (Amersham International plc, Amersham, Bucks) in order to calculate the exact volume of infusate which entered the isolated loop (necessary because of possible slight variations in infusion rate, leakages, etc.): this was done by relating the ^{51}Cr concentrations of the infusate and perfusate, having measured the volume of perfusate collected.

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Test solutions

Solutions of glucose or maltose (20 g/l; SAS Chemicals Ltd, London) were used, without or with guar gum (6·7 g/l; Meyprogat 150®, Meyhall Chemical (UK) Ltd, Wirral, Merseyside). The guar gum concentration was the amount calculated to be the average concentration in digesta in the jejunum, based on the amount used in the diet of our previous studies (60 g/kg) and the amount of digesta flowing through this region. The apparent viscosity of the solutions containing guar gum was 0·654 Ns/m^2 (654 cP) at 40° and a shear rate of 14 /s measured with a Haake Rotoviscometer (Model RV3; MSE Instruments Ltd, Manor Royal, Crawley, West Sussex). The N content of guar-gum-free infusate solutions was $<$ 10 mg/l, and 40 mg/l for those containing guar gum.

Analytical methods

The activity of ^{51}Cr in both the infusates and perfusates was counted for 2 min in an LKB-Wallac gamma-sample counter, Model 80000. The N content of the samples was measured by the Kjeldahl method.

Experimental plan and statistical analysis

During the first 4 d of the present study the jejunum of each pig was perfused with four solutions: Krebs–Ringer, Krebs–Ringer with glucose (20 g/l), new Ringer, new Ringer with glucose (20 g/l). Each solution was perfused for 6·5 h in each pig in a Latin square design.

During the following 4 weeks the pigs were perfused for 6·5 h on 3 d of each week. Each pig was perfused with each of the four test solutions (glucose or maltose, without or with guar gum) during the four successive weeks, in a Latin square design (using the new Ringer solution).

The effects of perfusing the loops of jejunum with the test solutions on net N secretion (after correction for the N content of the infusates) during the 6 h perfusion periods were compared by analysis of variance. In addition, the net amounts of N secreted during consecutive periods of 2 h (i.e. 1 and 2, 3 and 4, and 5 and 6) were compared individually, by analysis of variance, in order to test whether any significant changes in N secretion occurred during the perfusions.

RESULTS

Animal preparations

The animal preparations remained patent for 2 months after surgery. During this period there was negligible leakage from the cannulas and the general health and growth rate of the animals was good. No behavioural problems were encountered during the perfusions.

Effect of Ringer solution on N secretion

The results shown in Table 1 indicate that there were no significant differences in N secretion into the four test solutions used.

Effect of guar gum on N secretion

Table 2 shows the effect of guar gum on N secretion into solutions of glucose or maltose, with or without guar gum. Addition of guar gum to both the solutions of glucose and maltose significantly increased N secretion during the 6 h period. There were no significant differences between N secretion into the solutions of glucose and maltose.

The effects of guar gum on N secretion were only significant in hours 3 and 4, and 5 and 6. No significant differences in N secretion were found in any of the 2-h periods between the solutions of glucose and maltose.

Table 1. *Effects of Krebs–Ringer and new Ringer solutions* without or with glucose on nitrogen secretion (mg/m per 2 h) during 6-h perfusion periods in isolated loops of jejunum in pigs*

(Mean values for each 2-h period with their standard errors of differences between means for four pigs/treatment)

Solution	Period of perfusion (h)			
	1 and 2	3 and 4	5 and 6	1–6
Krebs–Ringer	69·3	91·7	61·4	74·1
Krebs–Ringer + glucose	90·2	117·9	94·5	100·9
New Ringer	76·1	85·6	78·3	80·0
New Ringer + glucose	77·9	93·3	113·9	95·0
SED	10·9	26·6	18·3	19·2

Comparison of N secretion over the 6-h period: SED 20·4 (for means of the same solution). There were no significant differences ($P > 0·05$) either between solutions or between different 2-h perfusion periods with the same solution.

* For details, see Table 2 of Rainbird *et al.* (1984).

Table 2. *Effects of guar gum on nitrogen secretion (mg/m per h) in isolated jejunal loops in pigs during 6-h perfusion periods (columns) and effects of time (rows)*

(Mean values for each 2-h period with their standard errors of differences between means for four pigs/treatment)

Solution	Period of perfusion (h)			
	1 and 2	3 and 4	5 and 6	1–6
Glucose	53·1	66·9	87·7	69·2
Glucose + guar gum	82·3[a]	162·8[b]	142·6[b]	133·9
	NS	***	**	***
Maltose	67·8	67·3	91·9	75·5
Maltose + guar gum	58·2[a]	155·2[b]	136·7[b]	120·2
	NS	**	*	**
Pooled SED for means at the same times (columns)	14·12	15·88	12·87	6·93
Pooled SED for means of same solution (rows) 22·07				

Differences in secretion between glucose and maltose solutions were not significant ($P > 0·05$).

[a,b] Within a horizontal row values with different superscript letters were significantly different ($P < 0·05$).

NS, not significant.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Effect of time on N secretion

The amounts of N secreted into the solutions of glucose and maltose without or with guar gum during the 6-h perfusion period are shown in Table 2. The mean amounts of N secreted were always higher in hours 3 and 4, and 5 and 6, than in hours 1 and 2, but these amounts were greater and only reached significance for the solutions containing guar gum.

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DISCUSSION

From a technical point of view, the animal preparations were very successful. While the use of an isolated loop can be criticized on the grounds that the motility of this region of the gut may have been disturbed by the presence of two re-entrant cannulas, this preparation worked well for a long period in conscious animals which grew normally. Such a preparation thus has several marked advantages over those using either in vitro or acute in vivo approaches for the study of absorptive function in the gut: the possibility of replicating several treatments in the same animal is of particular value. The perfusion rate of 480 ml/h was based on the flow-rate of digesta through the cannulas of the same pigs before the perfusion studies began. This contrasts with the rates of 720–800 ml/h or 1520 ml/h used by Buraczewska (1979) in similar preparations; at both flow-rates N secretion was similar, and these amounts were similar to those found in the present study. This suggests that the flow-rate chosen may not be too critical.

The lack of effect of the composition of the Ringer solution used suggests that the ionic concentrations within the gut lumen can vary over a considerable range without causing marked changes in N secretion.

The results suggest that the secretion of N into the isolated loops of jejunum did not reach a maximum until the third or fourth hour at least: although there were no statistically significant effects of time in the present study, this aspect of the perfusion technique may merit further study.

The question of the most appropriate dietary protein level for studies of the present type has been little investigated, although this appears to be of importance because Buraczewska (1979) found that N secretion into isolated loops of jejunum was halved when the pigs were fed on a protein-free diet after receiving a semi-purified diet containing 27·2 g N/kg. This suggests that the level of dietary protein is important in determining the rate of N secretion into the jejunum; it is of interest that this effect is clearly seen when the conditions for measuring N secretion into the loop of jejunum are identical, and in spite of the fact that in neither case is dietary protein passing through the loop. The results of the present study were obtained while pigs were consuming a diet containing 25·6 g N/kg.

Another technical question which may be asked in such studies concerns the type of perfusion medium to be used. The new Ringer solution used in the present study was prepared to resemble the ionic concentration of digesta in this part of the gut, but it did not contain the insoluble matter which is characteristic of normal digesta. Experience showed that our apparatus was not suitable for perfusion of digesta through the isolated loops of jejunum because there were continuous problems with blockage. However, Buraczewska (1979) perfused insoluble starch through loops of the ileum of pigs and found that there was slightly less secretion of N into this medium than into isotonic Krebs–Ringer solution. This suggests that the presence of insoluble material in suspension within the gut does not necessarily lead to increased N secretion; it may be that certain quite specific attributes of guar gum are associated with the effect it has in raising N secretion.

The amounts of N secreted into the loops when solutions without guar gum were infused, 830 and 906 mg/m per 24 h for solutions of glucose and maltose (20 g/l) respectively, were comparable with values of 970 and 1042 mg/m per 24 h obtained by Buraczewska (1979) when solutions containing 2 or 10 g glucose/l respectively, were infused. However, when Buraczewska (1979) infused a hypertonic solution of glucose (50 g/l), 1709 mg N/m per 24 h were secreted, compared with 1607 mg N/m per 24 h secreted into the glucose solution containing guar gum in the present study. Comparison of the osmolarity of the 50 g/l solution of glucose and the 20 g/l solution of glucose with guar gum showed the former

to have a much higher osmolarity (for details, see Rainbird *et al.* 1984). This suggests that osmolarity is not a primary determinant of N secretion into the jejunum in this experiment.

So far it is not possible to state the chemical nature of the increased N secretion into the jejunum, but preliminary studies indicate that this is probably at least partly in the form of protein and DNA (L.M.J. Heppell, personal communication). It is possible that guar gum caused an increase in mucosal cell losses in the gut lumen, as well as increased production of brush-border enzymes and mucoproteins. However, the reasons why such effects may have occurred are not known. It may be that the water-holding capacity of guar gum is one factor involved: 1 g guar gum may hold 21 ml water (Stephen & Cummings, 1979). This effect would tend to make the contents of the small intestine not only more viscous, but might possibly also increase their volume. Both factors could be important aspects of the effects seen in the present study. Whatever the detailed mode of action of guar gum within the small intestine, it is clearly multi-factorial because glucose absorption is reduced (Rainbird *et al.* 1984) at the same time as N secretion increases, and electromyographic measurements indicate modified patterns of gastro-duodenal motility (Rainbird, 1983).

Although no comparable measurements have been made on the effect of guar gum on N secretion into the small intestine, other changes in function have been seen following consumption of diets containing guar gum or pectin which also has the property of raising the viscosity of a meal. Jacobs (1983) found that addition of guar gum to a fibre-free diet for rats led to a 19% increase in mucosal cell mass, resulting from an increase in the length of the small intestine rather than in villus length; at the same time a higher rate of epithelial cell migration was seen in the rats fed on guar gum, leading to estimated villus cell transit times decreasing from 41·1 to 37·0 h. This would be expected to be associated with increased N secretion. Addition of pectin to the diet has been shown to cause hyperplasia of the gut in rats (Brown *et al.* 1979), increased mucosal cell turnover (Komai *et al.* 1982) and altered morphology and mucus production in rats (Cassidy *et al.* 1981). Each of these observations may provide possible explanations for the results of the present study.

Extrapolation of the results from the present study suggests that guar gum might lead to an increase in N secretion from the entire small intestinal mucosa (18 m) of a 40 kg pig from 15 to 27 g/d, assuming that the effect is constant throughout the small intestine. At present it is not possible to say whether the amounts of N secreted into the solutions of glucose or maltose alone or into the solutions with added guar gum more closely resemble the amounts secreted into the gut when digesta passes through it. However, the results do suggest that guar gum, and perhaps other types of dietary fibre, may be determinants of the amounts of N secreted into the small intestine.

The potential importance of such effects in whole-body N metabolism is shown by the results of Reeds *et al.* (1980), which indicate that the rate of protein synthesis in the pigs used in the present study would have been approximately 100 g/d (expressed as N). The importance of the small intestine in N metabolism was also shown by Simon *et al.* (1983) who found fractional rates of protein synthesis in different parts of this organ ranging from 30 to 304%/d: in the jejunum the range lay between 74 and 161% /d and in the jejunal mucosa between 71 and 117%/d. Assuming that the N content of the small intestine of the 40-kg pigs in the present study was 23·4 g (Edmunds *et al.* 1978) and the mean fractional synthetic rate was 104%/d (Simon *et al.* 1983), the calculated daily protein synthesis would have been 24·3 g. In spite of the many technical difficulties in estimating rates of protein turnover this value is consistent with the results of the present study. It should be noted that not all of the N secreted into the jejunum is necessarily the result of protein synthesis in this tissue. It is possible that urea and plasma proteins are secreted here, and some of the N from shed cells would be in the form of DNA. It is clear that more research is needed

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in this area before reliable quantitative information on this topic can be provided. However, these preliminary findings are of interest in the light of current emphasis on increasing the proportion of dietary fibre in the human diet.

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