The influence of diet on the exocrine pancreatic secretion of growing pigs

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1. Pancreatic juice was collected from six pigs of 48 kg initial weight fitted with a collection catheter in the pancreatic duct and a return catheter in the duodenum.

2. Measurements of flow and composition of the juice were made during 24 h periods after adaptation to isonitrogenous diets based on barley, wheatings and fish meal (diet BWF) or starch, sucrose, casein, maize oil and cellulose (diet SSC), given in a change-over design. Measurements were also made during the periods of adaptation to a change from one diet to the other.

3. Mean flow-rates for pigs adapted to diets showed a highly significant four-fold difference between diets; values were 4962 ml/d for diet BWF and 1273 ml/d for diet SSC. The hourly volumes of juice were very variable and showed no clear response to feeding and no consistent diurnal pattern for either diet.

4. There were no significant differences between diets in the specific activities of the proteases. Average values were (units/mg protein) trypsin (*EC* 3.4.21.4) 29·6, chymotrypsin (*EC* 3.4.21.1) 7·7, carboxypeptidase A (*EC* 3.4.17.1) 0·65, carboxypeptidase B (*EC* 3.4.17.2) 1·6. The total output of each tended to be higher with diet BWF than with diet SSC. The specific activities and total outputs of α-amylase (*EC* 3.2.1.1) and lipase (*EC* 3.1.1.3) were significantly higher for diet BWF than for diet SSC; specific activities for the two diets respectively were: (units/mg protein) α-amylase 95·6 and 42·3, lipase 59·0 and 14·5.

5. The higher daily volume of juice with diet BWF was associated with significantly (but only slightly) higher levels of both sodium and potassium, compared with diet SSC.

6. The results are discussed in relation to previous studies on digestion at this Institute, in which pigs with intestinal cannulas were given the same diets.

The importance of pancreatic juice in the digestive processes of the growing pig has been emphasized by studies in which the pancreatic duct has been ligated. Corring & Bourdon (1977) found that pigs with ligated pancreatic ducts grew at 421 g/d compared with 560 g/d for sham-operated controls. Ligation reduced the apparent digestibility of nitrogen by 35% and that of energy by 12%. Similar results have been reported by Pekas *et al.* (1964) and by Anderson & Ash (1971).

Chronic cannulation of the pancreatic duct has been used in several studies of pancreatic exocrine secretion in the pig. Pekas *et al.* (1966) studied the characteristics of secretion in pigs of 3–7 weeks of age. In older, growing pigs. Corring and his associates have made a number of studies including the secretory response to meal ingestion (Corring *et al.* 1972); the adaptation of secretion to different dietary protein levels (Corring & Saucier, 1972); feedback regulation of pancreatic secretion (Corring, 1974) and the influence of duodenal loads of sugars (Corring & Sumoes-Nunes, 1977).

One objective of the present study was to obtain further information on the influence of diet composition on the secretion of pancreatic juice and its components. The second objective was to resolve questions arising from previous work at Shinfield on digestive function in growing pigs. In recent years we have studied the processes of digestion and absorption by the technique of re-entrant cannulation at different sites in the small intestine. Reports have been published on the general characteristics of digesta flow and pH (Braude

et al. 1976); the flow of dry matter (DM) ash and water (Low *et al.* 1978); the passage and absorption of individual mineral nutrients (Partridge, 1978 *a*), nitrogen and amino acids (Low, 1979 *a*, *b*), carbohydrates (Sambrook, 1979 *a*), fibre and lipid (Sambrook, 1979 *b*). Throughout this work it has been apparent that considerable difficulties of interpretation arise from the fact that the re-entrant cannulation technique does not allow differentiation between dietary and endogenous components of the digesta samples collected. In particular, duodenal digesta in our studies, as in most of those elsewhere, has included bile and pancreatic juice mixed with the gastric effluent. This is due to the difficulty of placing re-entrant cannulas anterior to the bile and pancreatic duct openings, which in the pig are approximately 40 and 100 mm respectively caudal to the pylorus. Therefore, studies were initiated on the quantitative and qualitative contributions of bile and pancreatic juice to the duodenal digesta. This account concerns only pancreatic secretion; our work on bile secretion has been reported elsewhere (Sambrook, 1981). The same diets were used in these studies as in the digestion studies cited previously and the results will be discussed in relation to our observations on duodenal digesta. A brief report of part of this work has been published previously (Partridge *et al.* 1979).

EXPERIMENTAL

Six pigs of 48 kg, average initial live weight were prepared for complete collection and subsequent return of the pancreatic exocrine secretion, by the surgical procedure described by Corring *et al.* (1972). This involved the insertion of a polyethylene catheter into the pancreatic duct immediately above its point of entry into the duodenum. The duct was then tightly closed around the catheter by a suture. A second catheter was inserted into the duodenum, close to the normal point of entry of pancreatic juice. Both catheters were exteriorized through stab wounds in the flank. For a few days before surgery and during the recovery and experimental periods the pigs were kept in metabolism crates.

Throughout the trial, pancreatic juice was collected continuously by free drainage into a polyethylene bottle under ice. The juice was also returned continuously from a second container by means of a peristaltic pump. The pump was adjusted frequently so that the rate of juice return to the duodenum was similar to the rate of secretion.

Two isonitrogenous diets were compared: a practical growing pig diet based on barley, wheatings and fish meal (diet BWF) and a purified diet based on starch, sucrose and casein (diet SSC). The composition of these is given in Table 1. The average diet consumption during the experimental period was 1·6 kg/d. The animals were fed twice daily at 09.00 and 15.00 hours and the diet allowance was mixed with water just before feeding, allowing 2·5 l/kg diet. Each pig received both diets in a change-over design.

After recovering from surgery the animals were allowed a minimum of 5 d to become adapted to one or other of the test diets. Then flow rate was measured and samples were taken during two 24 h collections from each pig on consecutive days. Immediately following the second collection, each pig was abruptly changed onto the other test diet and two further consecutive 24 h collections were conducted, starting immediately, to monitor the changes in the characteristics of pancreatic secretion during the period of adaptation to the new diet. There were two further 24 h collections from each pig on days 5 and 6 following the diet change, when the pigs were deemed to have adapted to the new diet.

Collections started at 09.00 hours when the animals were given their morning feed. During the first collection from each pig when adapted to each of the diets, flow was measured and samples taken hourly throughout the 24 h period. For all remaining collections hourly measurements were recorded only until 21.00 hours, followed by a single measurement for the night period from 21.00 to 09.00 hours. At each of the stated times the juice collected in the bottle under ice was transferred to a measuring cylinder, the volume recorded and

Table 1. *Composition of experimental diets (g/kg)*

Diet BWF		Diet SSC	
Barley meal	712·5	Maize starch	612·7
Fine wheat offal	200·0	Sucrose	100·0
White-fish meal	70·0	Maize oil	30·0
NaCl	2·7	Cellulose	30·0
CaHPO₄.2H₂O	5·6	Casein	184·0
CaCO₃	6·2	CaHPO₄.2H₂O	20·6
Vitamin supplement*	2·0	CaCO₃	4·6
CuSO₄.5H₂O	1·0	NaCl	5·0
		Vitamin and trace mineral supplement*	13·1

* For composition, see Braude *et al.* (1976).

Table 2. *Substrates and conditions of analysis for the enzymes*

Enzyme	Substrate	Buffer	pH
Trypsin (*EC* 3.4.21.4)	*p*-Toluene-sulphonyl-L-arginine methyl ester	Tris-HCl, 0·046 M	8·1*
Chymotrypsin (*EC* 3.4.21.1)	Benzoyl-L-tyrosine ethyl ester	Tris-HCl, 0·08 M	7·8*
Carboxypeptidase A (*EC* 3.4.17.1)	Hippuryl-L-phenylalanine	Tris-HCl, 0·025 M	7·5†
Carboxypeptidase B (*EC* 3.4.17.2)	Hippuryl-L-arginine	Tris-HCl, 0·025 M	7·65‡
α Amylase (*EC* 3.2.1.1)	Soluble starch	Phosphate, 0·02 M	6·9§
Lipase (*EC* 3.1.1.3)	Stabilized olive oil emulsion	Tris-HCl, 0·20 M	7·7‖

* Hummel (1959). ‡ Folk *et al.* (1960). ‖ Tietz & Fiereck (1966).
† Folk & Schirmer (1963). § Bernfield (1951).

a 50 ml/l sample taken. For each pig the samples were bulked to form three samples representative of the first 6 h, second 6 h and last 12 h of each collection. These were stored at 4° to await analysis. After each sampling the remaining pancreatic juice was transferred to the return vessel from which it was pumped into the duodenum.

Each of the three composite samples of juice from each 24 h collection was analysed. With the exception of cation determinations which were done later on thawed samples, all measurements were done on fresh samples and were completed within 8 h of the end of the collection. Total protein was measured by the method of Lowry *et al.* (1951) and total N by the Kjeldahl method. Enzyme activities were measured by the procedures summarized in Table 2. For each enzyme, one unit of activity was defined as that which hydrolysed one μmol substrate/min per mg protein at 25°. The bicarbonate concentration was determined by boiling briefly with an excess of 0·1 M-hydrochloric acid and titrating the excess acid with 0·1 M-sodium hydroxide using phenolphthalein to determine the end-point. A silver electrode chloride meter (Evans Electroselenium Ltd) was used to measure chloride. Measurements of sodium and potassium were by flame emission and of calcium and magnesium by atomic absorption spectroscopy, performed on appropriate dilutions of the juice.

RESULTS

Considerable practical difficulties were encountered during the course of this trial. Only three pigs completed all planned collections, due to the premature loss of the pancreatic duct catheter from the remaining animals. Additional pigs were prepared but these were even less successful. This low rate of success at Shinfield is difficult to explain in view of the fact that one of the authors (T. Corring) has had consistent success with pigs prepared in a similar way at Jouy-en-Josas.

In the main, the results presented here concern the two 24 h collections planned with each of the six pigs after adaptation to each of the two diets. Eight of the twenty-four values (four for each diet) were missing and calculated values were inserted in the analyses of variance.

To summarize the results for collections during the periods of adaptation to diet changes, within 48 h the pigs appeared to be fully adapted to a change from diet BWF to diet SSC and were almost completely adapted to a change from diet SSC to diet BWF, in terms of flow-rate. For example, one pig when adapted to diet BWF produced 5598 ml juice/d; when changed abruptly to diet SSC the daily volumes were 3298, 1362 and 1396 ml for days 1, 2 and 5 respectively. Another pig initially adapted to diet SSC produced 1686 ml juice/d; when changed to diet BWF the daily volumes were 1930, 3881 and 5056 ml for days 1, 2 and 5 respectively. Values for individual components of the juice were very variable but in general these too showed a marked adaptation within 2 d of a change in diet.

There appeared to be no consistent differences in juice composition between the samples representing the three parts of the 24 h collections. The results were therefore combined to give single 24 h values for each constituent.

The flow of pancreatic juice

Mean values for the volume of pancreatic juice secreted in 24 h by pigs adapted to each of the two diets are given in Table 3. These results show that the volume with diet BWF was some four times that with diet SSC. Individual results within diets were quite variable but nevertheless the difference was highly significant. The mean hourly volumes of juice are shown in Fig. 1; individual values showed considerable variability and there was no clear response to feeding and no consistent diurnal pattern.

Enzyme composition

Mean values for the total protein content of the juice and the specific activities of the pancreatic enzymes are given in Table 3. Values for total N are also given to allow comparison with previous measurements in duodenal digesta. On average, the total weight of secreted protein was approximately 45% higher with diet BWF than with diet SSC. However, the individual values were very variable and the difference was not statistically significant. The specific activity values for each of the proteases and peptidases were very similar for the two diets. However, since there was a tendency for higher total protein secretion with diet BWF there was a tendency also for higher total outputs of the enzymes with this diet. The total outputs of amylase and lipase were considerably higher with diet BWF than with diet SSC due to the combined effects of significantly higher specific activities and the trend for higher total protein secretion.

Electrolyte composition

The anion and cation concentrations are given in Table 3. For both diets HCO_3^- was the predominant anion but with diet BWF the HCO_3^-:Cl^- value was significantly higher than with diet SSC. The concentrations of both Na and K were slightly, but significantly, higher with diet BWF than with diet SSC; for Ca and Mg, which made only small contributions

Table 3. *Flow and composition of pancreatic juice in 24 h collections from six pigs adapted to diets BWF and SSC† in a change-over design*

(Mean values for two collections from each pig on each diet, and the standard error of the difference (SED) between diet means: missing values were calculated for eight of the twenty-four plots)

	Diet BWF	Diet SSC	SED (2 df)
Total flow (ml)	4962	1273	182·6**
Total protein (g)	9·8	6·7	2·45 NS
Total nitrogen (g)	2·3	1·3	0·39 NS
	Enzyme specific activities (units/mg protein)		
Trypsin	32·0	27·1	2·55 NS
(*EC* 3.4.21.4)			
Chymotrypsin	7·6	7·8	2·69 NS
(*EC* 3.4.21.1)			
Carboxypeptidase A	0·6	0·7	0·21 NS
(*EC* 3.4.17.1)			
Carboxypeptidase B	1·7	1·5	0·38 NS
(*EC* 3.4.17.2)			
Amylase	95·6	42·3	1·91***
(*EC* 3.2.1.1)			
Lipase	59·0	14·5	12·68*
(*EC* 3.1.1.3)			
	Electrolyte composition (mmol/l)		
Chloride	19·1	33·9	4·16*
Bicarbonate	137·7	122·6	3·32*
Sodium	161·1	156·3	0·93*
Potassium	5·0	4·5	0·16*
Calcium	0·45	0·7	0·11 NS
Magnesium	0·1	0·2	0·03*

NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
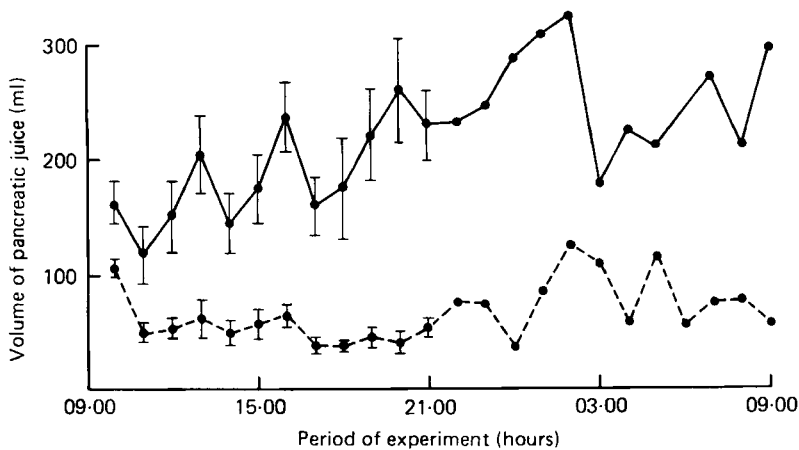† For details of diets, see Table 1.

Fig. 1. Hourly pattern of pancreatic juice flow according to diet composition: (●—●) diet BWF; (●---●) diet SSC (for details of diet, see Table 1). From 10.00 until 21.00 hours values are means of eight collections with their standard errors represented by vertical bars. From 22.00 until 09.00 hours values are means of two collections. The pigs were fed at 09.00 and 15.00 hours.

to the total cation concentration, the situation was reversed. The difference between diet means for Ca just failed to reach significance ($0.10 > P > 0.05$). The total amount of each electrolyte secreted was greater for diet BWF than for diet SSC by virtue of the greater volume of juice produced.

DISCUSSION

Volume and flow pattern of pancreatic juice

Corring (1980) reported that the average volume of pancreatic juice secreted in 24 h was 2·5 l for pigs of 45 kg live weight given a cereal diet. In the present study the volumes secreted by 48 kg pigs were approximately twice this value for a cereal diet (diet BWF) and half for a purified diet (diet SSC). In a recent study by Zebrowska et al. (1981) pancreatic juice was collected from 40 kg pigs prepared with short pouches of duodenum into which the pancreatic duct opened; daily volumes were 2·2 l for a cereal diet and 1·2 l for a purified diet. Their purified diet was very similar in composition to diet SSC and, indeed, both induced similar volumes of pancreatic juice. However, their cereal diet was based on barley and soya-bean meal in contrast to diet BWF (barley, wheatings and fish meal). Although in comparing these studies the effects of pig weight and collection technique are confounded with diet effects, it is evident that diet composition can have a marked effect on the daily volume of pancreatic juice secreted.

It is of interest to consider the volume of pancreatic juice in relation to those of duodenal digesta and bile, which have been studied in previous work at this Institute, using the same diets. In pigs with re-entrant duodenal cannulas, the flow of water through the duodenum in 24 h was 11·1 l and 7·1 l more than was ingested, for diets BWF and SSC respectively; DM flow was similar to intake (Low et al. 1978). The present results show that pancreatic juice contributed approximately 45 and 18% of these volumes of endogenous fluid, for diets BWF and SSC respectively. Studies reported by Sambrook (1981) showed that bile secretion in 40 kg pigs amounted to 1·7 l/d for diet BWF and 1·2 l/d for diet SSC. By difference, it can be calculated that the combined volume of saliva and gastric juice was similar for both diets at approximately 4·5 l/d. This value is of course nett of any reabsorption which may have occurred anterior to the proximal duodenum.

It was suggested by Low et al. (1978) that the differences in the water content of digesta in the duodenum, jejunum and ileum, and in faeces, were due to the higher fibre content of diet BWF (72 g acid-detergent fibre (ADF)/kg) compared with diet SSC (30 g ADF/kg). It is well known that dietary fibre increases intestinal water space in the pig (Cooper & Tyler, 1959; Farrell & Johnson, 1970; Partridge, 1978b). The present results and those of Sambrook (1981) suggest that the effects of fibre may be mediated through increased secretion of pancreatic juice and bile.

In agreement with the work reviewed by Corring (1980) the present results show that the secretion of pancreatic juice is continuous in the pig (Fig. 1). Corring et al. (1972) reported that pigs responded to feeding by increasing pancreatic juice protein secretion, which reached a maximum approximately 3 h after feeding; in some pigs this was achieved by increased protein concentration in the juice while in others there was an increase in hourly volume. In the present trial there appeared to be no increase in flow-rate after feeding with either diet; protein content of the juice was not measured on an hourly basis.

The daily secretion of total N in pancreatic juice was 2·3 g for diet BWF and 1·3 g for diet SSC. These amounts represent approximately 5·8 and 3·2% respectively of the total daily N throughput in the duodenum of cannulated pigs given similar amounts of the same diets in a previous study (Low, 1979a).

Enzyme content of pancreatic juice

The diets used differed considerably in their physical form; although their total protein content was similar, the types of protein differed: diet BWF contained a very wide variety of proteins while diet SSC contained only the relatively homogenous casein. These differences in protein type did not, however, lead to any significant change in the proportions of proteases or peptidases secreted. This observation was also made in pigs given similar diets by Zebrowska *et al.* (1981) in studies on pancreatic secretion, and by Low (1982) in studies on duodenal digesta. In common with the present study, the measurements of pancreatic proteases and peptidases were highly variable; the fact that this was found in three separate studies using different techniques suggests that this variability is a biological fact rather than a problem of analytical technique.

The proportions of chymotrypsin and trypsin secreted were similar to those found in pancreatic juice by Corring *et al.* (1972), and in duodenal digesta by Low (1982). However, the total amounts of activity differed between these three studies, probably due to differences in the level of feeding, diet composition or method of study.

The secretion of carboxypeptidases A and B found is generally similar to that obtained by Zebrowska *et al.* (1981), both in terms of total activity and proportions. In both studies carboxypeptidase A activity was less than half that of carboxypeptidase B, irrespective of diet.

The results of the present study indicate that synthesis and secretion of proteases greatly exceeds the amount which is theoretically required to hydrolyse dietary proteins.

Values for α-amylase and lipase showed an effect of diet on both specific and total activities. In the case of α-amylase, the results of the present study do not appear to agree with those of Corring (1980) who has shown that the secretion of an enzyme increases in response to an increased intake of its particular substrate: diet SSC in this study had a slightly higher content of starch than diet BWF but produced a lower secretion of α-amylase. However, the starch in diet SSC was purified maize starch, which is more readily digested and absorbed in the small intestine (Sambrook, 1979*a*) and produces a more pronounced blood glucose peak after feeding (Leeds *et al.* 1980) than the cereal starch in diet BWF. It may be that the more rapid release of starch hydrolysis products for diet SSC was responsible for the reduction in pancreatic α-amylase secretion for this diet.

It is more difficult to account for the reduced lipase secretion with diet SSC compared with diet BWF. Although diet BWF contained 25% more lipid than diet SSC, the lipid in the latter diet was in the form of pure maize oil which is better digested and absorbed in the small intestine than the lipid in the cereal diet (Sambrook, 1979*b*).

Electrolyte content of pancreatic juice

There appear to be no previous reports on the electrolyte content of pancreatic juice measured in conscious pigs under normal physiological conditions. Hickson (1970) reported that in anaesthetized pigs in which pancreatic secretion was stimulated by secretin injection or by vagal stimulation, HCO_3^- concentration rose from 20–30 mmol/l in resting juice to 130–160 mmol/l when a higher flow-rate was induced. Cl^- concentration responded in a reciprocal way, changing from 130 mmol/l in resting juice to 18–24 mmol/l at higher flow-rates. Similar responses to increasing flow-rate have been observed in other species (Janowitz, 1967). In the present study the same relationship was seen; the higher flow-rate associated with diet BWF, compared with diet SSC, was accompanied by a higher HCO_3^- and lower Cl^- concentration. However, in this trial the values for both diets were comparable with those for the higher rates of flow in anaesthetized pigs. It is not possible to compare flow-rates directly as those of Hickson (1970) were reported as μl/g pancreas per min.

The higher HCO_3^- content of pancreatic juice coupled with the higher flow-rate in pigs given diet BWF resulted in a marked increase in the total buffering capacity in comparison with diet SSC. Average values for the daily secretion of bicarbonate were 683 and 156 mmol for diets BWF and SSC respectively. In spite of this, previous measurements of the pH of duodenal digesta collected posterior to the pancreatic duct showed that digesta resulting from diet BWF was more acidic than that from diet SSC; average values over 24 h were 4·4 and 4·9 respectively (Braude *et al.* 1976). These results suggest that there was a much greater secretion of gastric acid with diet BWF.

The concentrations of Na and K in the present trial were similar to values for the anaesthetized pigs of Hickson (1970), who further reported that their concentrations did not change with increasing flow-rate induced by secretin or by vagal stimulation. The concentrations of both Na and K were significantly higher with diet BWF than with diet SSC but the difference was small numerically.

The total daily output of Na in pancreatic juice was 18·4 g for diet BWF and 4·6 g for diet SSC. Partridge (1978 a) reported that for pigs of similar weight given similar amounts of diet BWF and SSC the daily throughput of Na in the duodenum exceeded intake by 21·6 and 13·4 g respectively for the two diets. Thus pancreatic juice accounted for a large proportion of the endogenous Na and for a large part of the dietary difference. The previous duodenal studies also showed a daily net secretion of K of 3·1 g for diet BWF and 2·6 g for diet SSC; the present results show that pancreatic juice accounted for 1·0 and 0·2 g K/d for the respective diets.

The Ca and Mg contents of pancreatic juice were relatively small. For these, duodenal throughput was very similar to intake in our previous work (Partridge, 1978 a). The reason for the significant difference between diets in the Mg concentration in pancreatic juice is not readily apparent. The higher concentration of Mg and the tendency for a higher concentration of Ca with diet SSC may simply reflect the lower flow rate, compared with diet BWF. However, the total daily output of each of these elements was still considerably higher for diet BWF.

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