The activity of pepsin, chymotrypsin and trypsin during 24 h periods in the small intestine of growing pigs

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1. Digesta were collected from twenty-two pigs, of 40 kg mean live weight, and fitted with single re-entrant cannulas in either the duodenum, jejunum or ileum.

2. Three approximately isonitrogenous diets were given to the pigs; their main constituents were: barley, fine wheat offal and white fish meal (diet BWF); starch, sucrose, maize oil, cellulose and either groundnut (diet SSG) or casein (diet SSC).

3. The activities of pepsin, chymotrypsin and trypsin were measured every hour in duodenal digesta during 24 h collection periods. Chymotrypsin and trypsin were also measured every hour in jejunal digesta and every 6 h in ileal digesta, during 24 h collection periods.

4. The mean total pepsin activities in the duodenal digesta during 24 h collection periods (units for a 40 kg pig given 1·7 kg diet) were: 7764400 (diet BWF), 6078400 (diet SSG), 5801 600 (diet SSC).

5. The mean total chymotrypsin activities (units for a 40 kg pig given 1·7 kg diet) in digesta in the duodenum, jejunum and ileum respectively were: 62920, 59560, 21880 (diet BWF), 78240, 68400, 24680 (diet SSG), 75280, 76120, 6160 (diet SSC).

6. The mean total trypsin activities (units for a 40 kg pig given 1·7 kg diet) in digesta from the duodenum, jejunum and ileum, respectively were: 256840, 362840, 77600 (diet BWF), 211200, 205280, 46720 (diet SSG), 325720, 428560, 13600 (diet SSC).

7. It was calculated that the total weights of pepsin, chymotrypsin and trypsin in duodenal digesta in 24 h periods were between 6·2 and 7·1 g. This represents 20–25% of previously published estimates of the amounts of endogenous protein in this part of the gut.

In previous papers in this series the digestion and absorption of dietary dry matter, nitrogen and amino acids in the intestines of pigs with re-entrant cannulas have been described (Braude *et al.* 1976; Low *et al.* 1978; Low 1979*a*, *b*). During these studies the amounts of the proteases pepsin (*EC* 3.4.23.1), chymotrypsin (*EC* 3.4.21.1) and trypsin (*EC* 3.4.21.4) were measured with the objectives of assessing: (1) the total activities of these enzymes in 24 h collection periods, (2) the pattern of activity during 24 h collection periods, (3) whether activity was modified by different sources of dietary protein, (4) activity at three sites in the small intestine, and (5) whether measurements in duodenal digesta corresponded with measurements of chymotrypsin and trypsin in pancreatic juice.

Gastric secretion has been studied in pigs by means of isolating the stomach (Kvasnitskii, 1951; Kowalewski *et al.* 1974), stomach pouches (Kvasnitskii, 1951; Höller, 1970; Cranwell & Titchen, 1974, 1976) and in the intact stomach (Kvasnitskii, 1951; Hohlacev & Kasincev, 1962; Maxwell *et al.* 1970; Lawrence, 1972; Cranwell, 1977). However, none of these procedures has allowed estimates of the total amount of pepsin secreted by the stomach to be made; in the present study the amount of activity in the duodenum was used as a measure of the total secretion of pepsin.

The amounts of the pancreatic proteases chymotrypsin and trypsin have been measured after activation, in juice collected from pigs with catheters in the pancreatic duct (Corring *et al.* 1972; Corring & Saucier, 1972; Partridge *et al.* 1982). General protease activity in the pancreatic juice of pigs has also been measured (Kvasnitskii, 1951; Pekas *et al.* 1966; Tkachev *et al.* 1970). Measurements of the activity of these enzymes in the digesta of the small intestine of growing pigs have not been reported hitherto.

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

Ingredients	Diets BWF and BWF_f*		Diet SSG	Diet SSC
Barley meal	712·5	Maize starch	277·0	612·7
Fine wheat offal	200·0	Sucrose	276·9	100·0
White fish meal	70·0	Maize oil	30·0	30·0
NaCl	2·7	Solka Floc‡	20·0	30·0
$CaHPO_4 . 2H_2O$	5·6	Groundnut meal	350·0	—
$CaCO_3$	6·2	Casein	—	184·0
Vitamin mix no. 1†	2·0	Trace mineral mix§	10·0	10·0
$CuSO_4 . 5H_2O$	1·0	$CaHPO_4 . 2H_2O$	17·9	20·6
		$CaCO_3$	4·6	4·6
		Vitamin mix no. 2‖	2·0	2·0
		Choline hydrochloride	1·1	1·1
		NaCl	5·0	5·0
		L-lysine hydrochloride	2·5	—
		DL-methionine	3·0	—

* Diet BWF after milling through a 1 mm mesh; this diet was given to pigs with ileal re-entrant cannulas.

† Supplied (/kg diet): 0·75 mg retinol, 7·50 μg cholecalciferol, 3·25 mg riboflavin, 30·00 μg cyanocobalamin, 15·75 mg nicotinic acid, 13·00 mg pantothenic acid, 3·25 mg pyridoxine, 200·00 mg choline chloride, 2·00 mg DL-α-tocopheryl acetate.

‡ Brown & Co., Berlin, New Hampshire, USA.

§ Supplied (/kg diet): 4·47 g K_2CO_3, 1·73 g $MgCO_3 . H_2O$, 0·33 g $FeSO_4 . 7H_2O$, 60 mg $MnSO_4 . H_2O$, 0·10 g $ZnCO_3$, 8·00 mg NaF, 17·50 mg $CuSO_4 . 5H_2O$, 6·00 mg $CoCl_2$.

‖ Supplied (/kg diet): as vitamin mix no. 1 (omitting choline chloride) and in addition 2·00 mg thiamin, 50·00 μg biotin, 0·50 mg pteroylmonoglutamic acid, 20·00 mg *p*-aminobenzoic acid, 194·00 mg *myo*-inositol, 30·00 mg ascorbic acid, 2·00 mg menaphthone.

EXPERIMENTAL METHODS

Animals. Castrated male Large White pigs were used. Single re-entrant cannulas (Ash, 1962) were fitted as follows: (*a*) duodenum, approximately 0·15 m from the pylorus and distal to the bile and pancreatic ducts (eleven pigs); (*b*) jejunum, 2·0–2·5 m from the pylorus (i.e. 13–17% of the distance along the small intestine) (five pigs), or (*c*) ileum, 0·3 m from the ileo-caecal junction (i.e. more than 98% of the distance along the small intestine) (six pigs). The average live weight of the pigs during the trial was 40 kg.

Housing. Details of the housing and metabolism cage design were as described by Braude *et al.* (1976).

Diets. Diets BWF, SSG and SSC (for details, see Table 1) contained respectively, 23·7, 24·7 and 24·5 g N/kg diet (average values for the various batches used). Because of previous experience of frequent blockages of ileal re-entrant cannulas after feeding diet BWF (ingredients milled through a 3 mm mesh), this diet was finely milled through a 1 mm mesh (diet BWF_f); this diet was fed to all pigs with ileal re-entrant cannulas used in the present trials. The dry diet was mixed with water (1 : 2·5, w/v) and offered daily at 09.00 and 15.00 hours. The animals were weighed weekly and fed on a scale based on their live weight (Barber *et al.* 1972). On this scale 40 kg pigs received 1·7 kg air-dry diet/d. Pigs received each of the diets for approximately 14 d (including a 5 d change-over period between diets).

Cannula design, surgery and digesta collection procedures. These were as described by Braude *et al.* (1976). Digesta were collected in bottles surrounded by ice and water mixture to minimize digestion outside the animal.

Sample preparation. During 24 h collections samples of digesta collected from pigs with

duodenal or jejunal cannulas were removed every hour, and every 6 h from pigs with ileal cannulas. Immediately after collection the solid and liquid fractions of the digesta were separated by centrifugation at 2500 *g* for 15 min (Minor centrifuge; MSE Ltd, Crawley, Sussex). The liquid phase was then stored at −20° until analysis. Tests showed that negligible amounts of enzyme activity were present in the solid fraction of the digesta and that storage for up to 3 months at −20° did not affect the enzyme activities. Assays were normally made within 1 month of sample collection.

Enzyme assay

Pepsin. The method of Anson (1938) was followed. Samples of 0·1 ml digesta were incubated with 5 ml haemoglobin solution (Worthington Biochemical Corporation, Freehold, New Jersey, USA; 25 g/l hydrochloric acid, pH 2) at 37° for 10 min. The reaction was stopped by the addition of 10 ml of trichloroacetic acid (100 g/l). The contents of the tube were filtered (no. 541, Whatman Ltd, Maidstone, Kent) and the filtrate absorbancy was measured at 280 nm (Spectrophotometer model SP500; Pye Unicam Ltd, Cambridge) in 10 mm cells. The activity of the samples was compared with that of a purified preparation of porcine pepsin found to have 2500 units/mg protein (Sigma London Chemical Co., Poole, Dorset) in order to check assay conditions and to estimate the total weight of pepsin secreted. One unit of activity is defined as an absorbance increase of 0·001/min at 280 nm of trichloracetic acid-soluble hydrolysis products. Because preliminary tests showed that the addition of pepsin or HCl to digesta did not increase pepsin activity, it was assumed that all the pepsinogen had been converted to pepsin by the time it reached the duodenum. Pepsin activity was only measured in digesta from the duodenum.

Chymotrypsin. The method of Hummel (1959) was followed using n-benzoyl-L-tryosine ethyl ester (BTEE) as substrate (British Drug House Ltd, Poole, Dorset). Samples were diluted 1:9 with 0·001 M-HCl before assay. The assay was made using a double-beam continuous recording spectrophotometer (model CF4, Optica UK Ltd, Gateshead): 0·1 ml diluted sample was added to 1·4 ml 0·001 M-BTEE (made up in water–methanol (50:50 w/w)), and 1·5 ml 0·08 M-Tris buffer, pH 7·8, containing 0·1 M-calcium choride. The reaction was run for 3 min at 256 nm and 25° in 10 mm cells. The activity of the samples was compared with that of a purified preparation of bovine chymotrypsin found to have an activity of 44·2 units/mg protein (Sigma London Chemical Co. Ltd, Poole, Dorset) in order to check asssay conditions and to estimate the total weight of chymotrypsin secreted. One unit of activity is defined as the hydrolysis of 1 μmol substrate in 1 min at 25° and pH 7·8. Because preliminary tests showed that the addition of trypsin to digesta did not increase chymotrypsin activity, it was assumed that all of the chymotrypsinogen had been converted to chymotrypsin before analysis in every sample.

Trypsin. The method of Hummel (1959) was followed using α-N-toluene-*p*-sulphonyl-L-arginine methyl ester hydrochloride (TAME) as substrate (British Drug Houses Ltd, Poole, Dorset). The sample dilution and spectrophotometer were as for chymotrypsin assay. The reaction was run with 0·1 ml diluted sample, 0·3 ml 0·01 M-TAME and 2·6 ml 0·046 M-Tris buffer, pH 8·1, containing 0·0115 M-calcium chloride, at 247 nm for 3 min at 25° in 10 mm cells. The activity of the samples was compared with that of a purified preparation of bovine trypsin found to have an activity of 106·1 units/mg protein (Sigma London Chemical Co. Ltd, Poole, Dorset) in order to check assay conditions and to estimate the total weight of trypsin secreted. One unit of activity is defined as the hydrolysis of 1 μmol substrate in 1 min at 25° and pH 8·1. Because preliminary tests showed that the addition of enterokinase (1 mg/ml of 0·001 M-HCl) to digesta did not increase trypsin activity, it was assumed that all trypsinogen had been converted to trypsin before analysis in every sample.

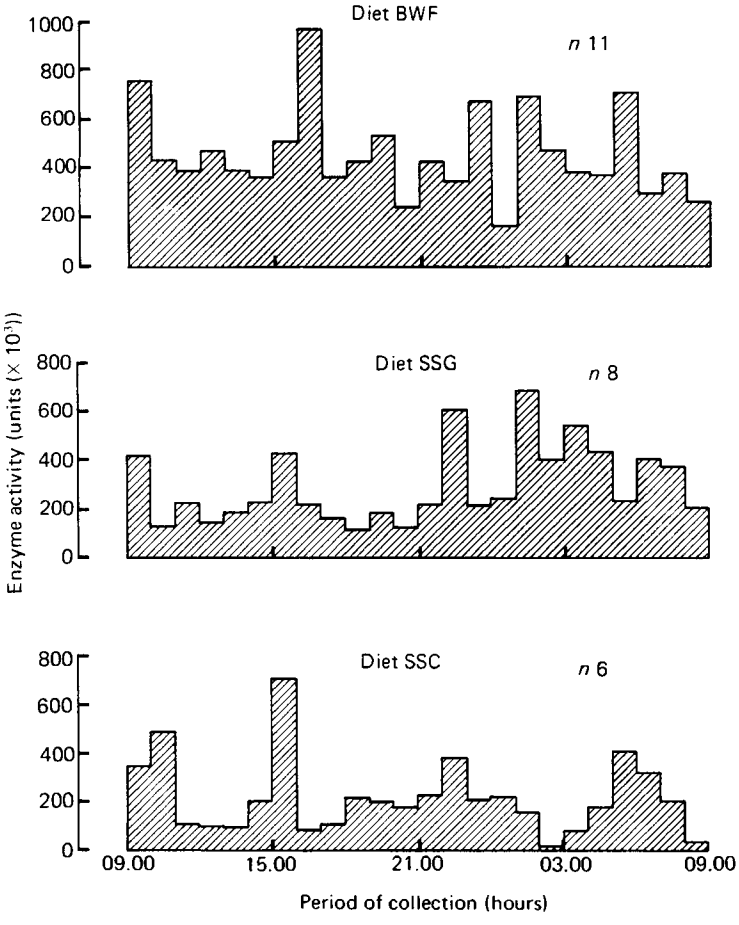

Fig. 1. Mean hourly activity of pepsin (*EC* 3.4.23.1) in the duodenum of 40 kg live weight pigs fitted with re-entrant cannulas and given successively diets BWF, SSG and SSC (for details, see Table 1). Pigs were fed at 09.00 and 15.00 hours. *n* is the number of pigs completing collections.

Table 2. *Activities of pepsin* (EC *3.4.23.1*) *during 24 h collection periods in pigs, with a single re-entrant cannula in the duodenum, given 1·7 kg diet*

(Mean values with their standard errors; 11 df; no. of pigs completing collections in parentheses)

Diet*	BWF (11)	SSG (8)	SSC (6)
Mean	7764400	6078400	5801600
SE	827920	970800	1120960

None of the differences between diet means were significant.
* For details of diets, see Table 1.

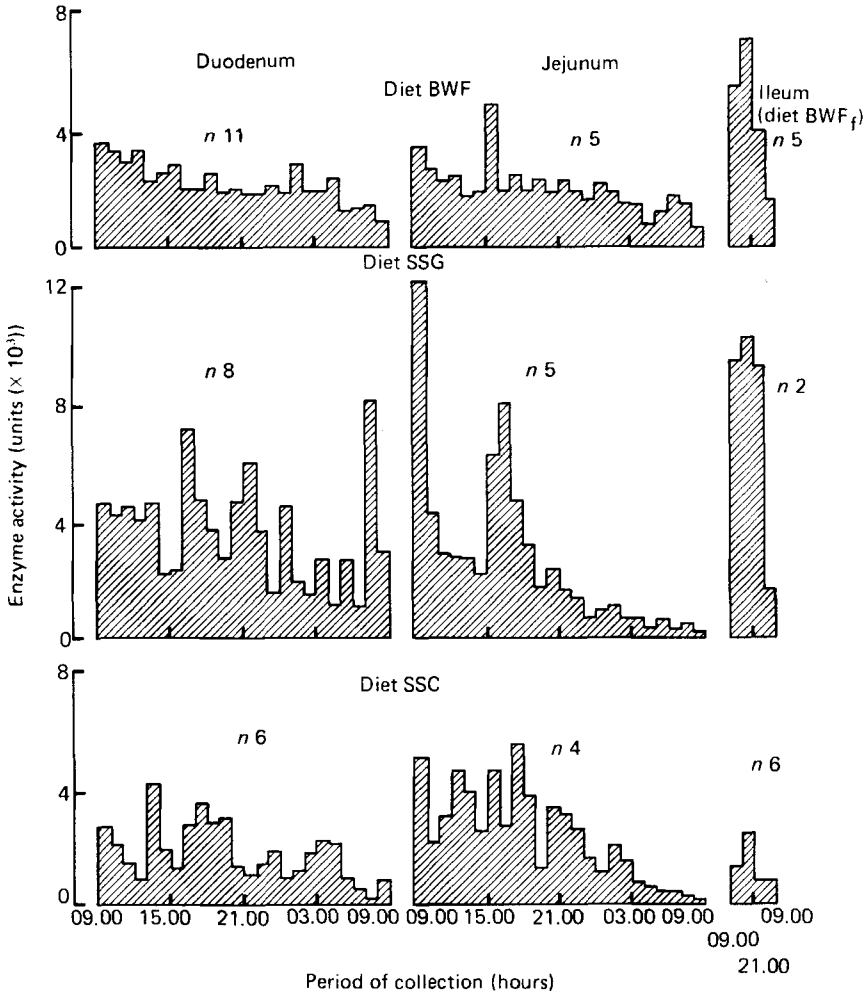

Fig. 2. Mean hourly activity of chymotrypsin (*EC* 3.4.21.1) (six-hourly in ileum) in 40 kg live weight pigs each fitted with a single re-entrant cannula (for details of sites, see p. 148) and given successively diets BWF (or BWF$_f$), SSG and SSC (for details, see Table 1). Pigs were fed at 09.00 and 15.00 hours. *n* is the number of pigs completing collections.

Presentation and statistical analysis of results

The total activities of the enzymes are expressed as units of activity measured in pigs of average live weight 40 kg. The pigs received 1·7 kg diet/d. It is assumed that protease secretion is directly related to live weight in the range used in this study. Thus values for pigs receiving between 1·5 and 1·9 kg (the lowest and highest values) have been adjusted to give equivalent values for an intake of 1·7 kg.

Activity pattern during 24 h periods. The mean values for the activities during 24 h collection periods were calculated from the average values for all 24 h collections completed by a pig on a diet. Pigs with duodenal and jejunal cannulas completed two 24 h collections per diet, while pigs with ileal cannulas completed four 24 h collections per diet. For pigs with

Table 3. *Activities of chymotrypsin* (EC *3.4.21.1*) *during 24 h collection periods in pigs, with single re-entrant cannulas in the duodenum, jejunum or ileum, given 1·7 kg diet*

(Mean values, and differences between the means with their standard errors for comparisons between sites and diets; no. of pigs completing collections in parentheses)

Diet†... / Site of re-entrant cannula§	BWF‡	SSG	SSC
Duodenum	62920(11)	78240(8)	75280(6)
Jejunum	59560 (5)	68400(5)	76120(4)
Ileum	21880 (5)	24680(2)	6160(6)

Comparisons of sites

Diet...	BWF		SSG		SSC	
	Difference	SE‖	Difference	SE‖	Difference	SE‖
Duodenum–jejunum	3320	20984	9840	22180	-840	25116
Duodenum–ileum	41040	20984	53560	30760	69120**	22464
Jejunum–ileum	37680	24608	43720	32552	69960*	25116

Comparsions of diets

Diet...	BWF–SSG		BWF–SSC		SSG–SSC		df
	Difference	SE¶	Difference	SE¶	Difference	SE¶	
Duodenum	15320	19160	12360	20672	2960	26004	11
Jejunum	8840	22352	16520	24360	7680	24364	7
Ileum	2800	5800	15720**	3584	18560*	5020	5

Levels of significance: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

† For details of diets, see Table 1.

‡ Diet BWF was finely-milled when given to pigs with ileal cannulas.

§ For details of sites, see p. 148.

‖ Based on the pooled error between-pigs-within-sites-and-diets.

¶ Based on the interaction between pigs and diets at an individual site (since there was some evidence of heterogeneity between sites) and an approximation (Taylor, 1948) when necessary.

duodenal or jejunal cannulas the mean values are for each hour of the 24 h periods and for those with ileal cannulas for the four successive 6 h periods of the 24 h periods.

Total activity in 24 h periods. The average activities for the whole of each 24 h period for each pig on a diet were compared by analysis of variance. The standard error of the difference between the means is not the same for each pair of cannula sites because different numbers of animals completed collections for the various site–diet combinations.

RESULTS

Although re-entrant cannulas were fitted to a total of thirty-five pigs during this study, results are only presented from twenty-two of these animals; problems were experienced with the remaining animals. These difficulties, which were described in detail by Braude *et al.* (1976), account for the unbalanced numbers of pigs used for the various site and diet combinations.

Pepsin. The mean hourly pattern of activity during 24 h is shown in Fig. 1. The coefficients of variation of the mean hourly values were very high with an average value of 93%. The mean activities of pepsin in 24 h periods are shown in Table 2. There were no significant differences between the diets in mean activities.

Chymotrypsin. The mean hourly pattern of activity (six-hourly in the ileum) is shown in Fig. 2. The coefficients of variation (%) of the mean hourly values (six-hourly values in the

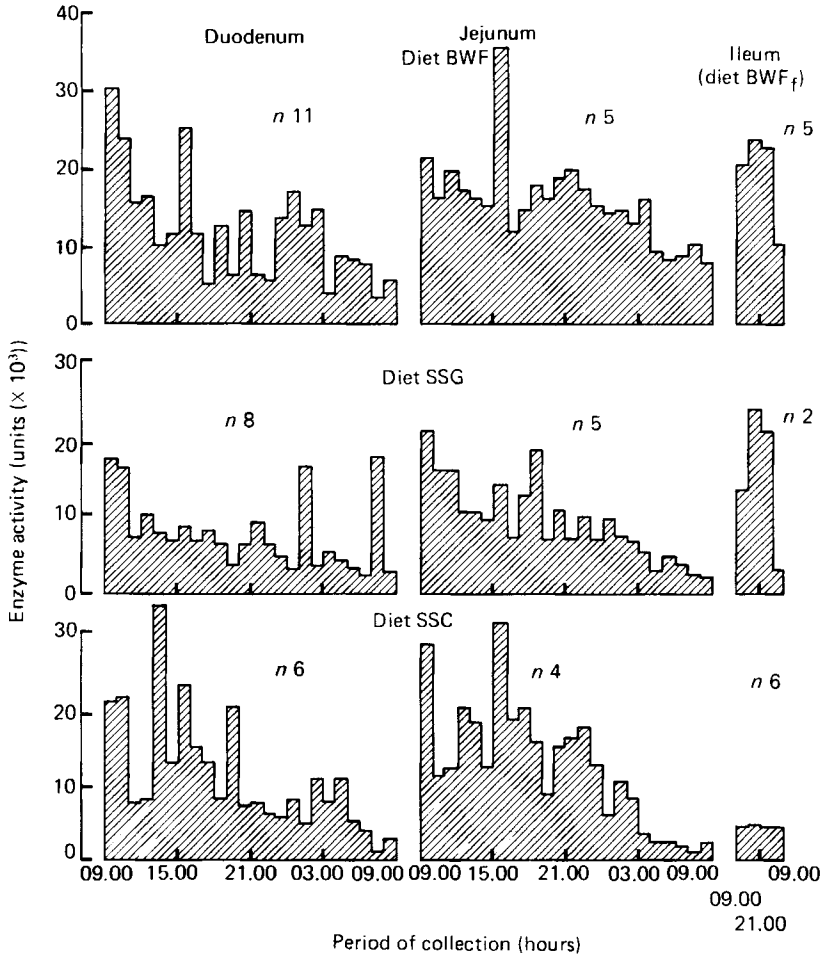

Fig. 3. Mean hourly activity of trypsin (*EC* 3.4.21.4) (six-hourly in ileum) in 40 kg live weight pigs each fitted with a single re-entrant cannula (for details of sites, see p. 148) and given successively diets BWF (or BWF_f), SSG and SSC (for details, see Table 1). Pigs were fed at 09.00 and 15.00 hours. *n* is the number of pigs completing collections.

ileum) were: duodenum 48, jejunum 55, ileum 37. The mean activities of chymotrypsin in 24 h periods are shown in Table 3. The high level of variability is notable. The only significant between-diet differences in activity were for the terminal ileum where the values for diet SSC were lower than those for diets BWF and SSG. The only significant between-site differences in activity were for diet SSC where the ileal value was lower than those for the duodenum ($P < 0.01$) or jejunum ($P < 0.05$).

Trypsin. The mean hourly pattern of activity (six-hourly values in the ileum) is shown in Fig. 3. The coefficients of variation (%) of the mean hourly values (six-hourly values in the ileum) were: duodenum 96, jejunum 34, ileum 33. The mean activities of trypsin in 24 h periods are shown in Table 4. As with chymotrypsin, the high level of variability is notable. Only one of the between-diet differences was significant (in the ileum, for diets BWF and SSC, $P < 0.01$). The ileal acitivity was significantly lower than at the duodenum ($P < 0.05$)

Table 4. *Activities of trypsin* (EC *3.4.21.4*) *during 24 h collection periods in pigs with single re-entrant cannulas in the duodenum, jejunum or ileum, given 1·7 kg diet*

(Mean values and differences between means with their standard errors for comparisons between sites and diets; no. of pigs completing collections in parentheses)

Diet...† / Site of re-entrant cannula§	BWF‡	SSG	SSC
Duodenum	256480(11)	211200(8)	325720(6)
Jejunum	362480 (5)	205280(5)	428560(4)
Ileum	77600 (5)	46720(2)	13600(6)

Comparisons of sites

Diet...	BWF		SSG		SSC	
	Difference	SE‖	Difference	SE‖	Difference	SE‖
Duodenum–jejunum	−106230	66424	5920	70208	−102840	79496
Duodenum–ileum	178920*	66424	164480	97360	312120***	71104
Jejunum–ileum	285240**	77888	158560	103036	414960***	79496

Comparisons of diets

Diets...	BWF–SSG		BWF–SSC		SSG–SSC		df
	Difference	SE¶	Difference	SE¶	Difference	SE¶	
Duodenum	45320	43672	69200	47100	114520	59256	11
Jejunum	157560	99508	65720	108436	223280	108436	7
Ileum	30880	20824	64000**	12868	33120	18036	5

Levels of significance: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

† For details of diets, see Table 1.

‡ Diet BWF was finely-milled when given to pigs with ileal cannulas.

§ For details of sites, see p. 148.

‖ Based on the pooled error between-pigs-within-sites-and-diets.

¶ Based on the interaction between pigs and diets at an individual site (since there was some evidence of heterogeneity between sites) and an approximation (Taylor, 1948) when necessary.

Table 5. *Calculated weights of pepsin* (EC *3.4.23.1*), *chymotrypsin* (EC *3.4.21.1*) *and trypsin* (EC *3.4.21.4*) *in digesta during 24 h collection periods in pigs with re-entrant cannulas*

(Values based on comparisons of activity in digesta and activity of purified enzymes [g/24 h for a pig of 40 kg live weight])

Enzyme	Site of re-entrant cannula*	Diet BWF†	Diet SSG	Diet SSC
Pepsin	Duodenum	3·1	2·4	2·3
Chymotrypsin	Duodenum	1·4	1·8	1·7
	Jejunum	1·3	1·5	1·7
	Ileum	0·5	0·6	0·1
Trypsin	Duodenum	2·4	2·0	3·1
	Jejunum	3·4	1·9	4·0
	Ileum	0·7	0·4	0·1

• For details of sites, see p. 148.

† Diet BWF was finely milled (diet BWF_f) when given to pigs with ileal cannulas; for details, see Table 1.

Table 6. *Calculated values for weights of chymotrypsin* (EC *3.4.21.1*) *and trypsin* (EC *3.4.21.4*)*: weight of amino acids in digesta* (*Low, 1979*b) *during 24 h collections from pigs with re-entrant cannulas*

Site of re-entrant cannula*	Diet BWF†	Diet SSG	Diet SSC
		Chymotrypsin	
Duodenum	0·007	0·008	0·006
Jejunum	0·008	0·008	0·008
Ileum	0·012	0·015	0·007
		Trypsin	
Duodenum	0·011	0·009	0·010
Jejunum	0·021	0·011	0·020
Ileum	0·017	0·010	0·007

* For details of sites, see p. 148.
† Diet BWF was finely milled (diet BWF_f) when given to pigs with ileal cannulas; for details see, Table 1.

Table 7. *Calculated values for chymotrypsin* (EC *3.4.21.1*)*: trypsin* (EC *3.4.21.4*) *by weight in digesta during 24 h collection periods in pigs with re-entrant cannulas*

Site of re-entrant cannula*	Diet BWF†	Diet SSG	Diet SSC
Duodenum	0·583	0·900	0·548
Jejunum	0·382	0·789	0·425
Ileum	0·714	1·500	1·000

* For details of sites, see p. 148.
† Diet BWF was finely milled (diet BWF_f) when given to pigs with ileal cannulas; for details, see Table 1.

and jejunum ($P < 0·01$) for diet BWF, and at both the duodenum and jejunum ($P < 0·001$) for diet SSC: the lack of significance for diet SSG may have been due in part to the fact that this diet was only represented by two pigs in the ileum.

Calculated weights of enzymes in digesta. The activities have been compared with the activities of the most highly purified preparations commercially available of pepsin, trypsin and chymotrypsin to obtain approximate estimates of the amounts of enzyme proteins in the digesta in 24 h periods: these are shown in Table 5.

Calculated weight of enzymes in digesta: weight of amino acids in digesta. The weights shown in Table 5 have been expressed relative to the total weight of the substrate protein in the digesta (as total amino acids; Low, 1979*b*) during 24 h collections; the values obtained are shown in Table 6.

Calculated chymotrypsin: trypsin in digesta. The relative weights of these enzymes are shown in Table 7.

DISCUSSION

General. Measurements of enzyme activity in digesta are open to criticism if one wishes to make an estimate of the original amount of enzyme secreted because of the possible inhibition by diet components, enzyme binding to insoluble diet components and because of denaturation or autodigestion. However, these problems appear not to have been of great

importance for chymotrypsin and trypsin because the results obtained were similar to those obtained by Partridge *et al.* (1982) in a study with pigs fitted with catheters in the pancreatic ducts and given diets BWF and SSC. It is probable that autodigestion and denaturation were limited by the large amounts of soluble protein and peptide in the digesta (Low, 1979*a*) which are known to exert a protective action on the active site of enzymes (Determan *et al.* 1969).

Although the method of collecting digesta can influence the flow of dry matter and N in the duodenum during 24 h periods, the amount of soluble digesta, in which the proteases were found, does not seem to be affected (Low & Zebrowska, 1977).

A notable feature of the studies is the very high level of variability of activity; however, enzyme activity measurements in pancreatic juice collected from pigs given diets BWF and SSC were also very variable (Partridge *et al.* 1982).

The 5 d change-over period between diets used in this study should have been sufficient to allow any enzyme adaptation to be completed. Corring & Saucier (1972) found that the pancreas adapted to large changes in protein intake by growing pigs within 2–3 d.

The estimates made of total weights of enzyme protein should be viewed with caution in view of the fact that the commercial preparations used contained traces of impurities and that commercial preparations of porcine chymotrypsin and trypsin were not available: the bovine enzymes used have slightly different biochemical characteristics from the porcine forms. Since the trial was completed the bovine enzymes have been compared with newly-available porcine forms which were found to have virtually identical activities in both instances.

Pepsin. Although haemoglobin, which was used for pepsin assay, is a non-specific substrate for proteases, the assay was performed at pH 2 i.e. below the pH at which the intestinal proteases and peptidases are active. Several forms of pepsin are known to exist; the activity measured here is the combined effect of all forms. Although pepsin is slowly and irreversibly denatured above pH 6 (Bovey & Yanari, 1960), this would have been of minor importance in the present study because the pH in the duodenal digesta seldom reached this value (Braude *et al.* 1976), and when it did there was little dry matter flow and so it probably contained little digesta of gastric origin (Low *et al.* 1978). Since the pH of the duodenal digesta was frequently below 5·5 (Braude *et al.* 1976) and thus in the range in which pepsin has marked activity while the pancreatic proteases have very little activity, some peptic proteolysis probably occurs in the duodenum of pigs. In general, as the pH of the digesta increased so the total amount of pepsin in the digesta decreased, probably because little of the digesta was of gastric origin during these periods.

There was often high pepsin activity during the night; this is consistent with observations made in pigs with isolated gastric pouches by Kvasnitskii (1951) and Höller (1970), but why the amount of activity was high when the stomach contained relatively little protein from the diet is not clear.

Chymotryspin. The pattern of activity during 24 h periods in the duodenum was somewhat similar to that found by Corring *et al.* (1972) who noted a response to feeding, continuity during the night (but at a lower level than by day) and some indication of an increase before feeding.

The mean total chymotrypsin activity in 24 h in digesta was approximately twice that reported by Corring *et al.* (1972). However, Corring *et al.* (1972) fed their pigs at approximately half the level used in the present study. Comparison of the present values and those of Corring *et al.* (1972) has been made after taking into consideration the fact that the latter authors used acetyl tyrosine ethyl ester as substrate which has a k_0 value (no. molecules transformed/per molecule enzyme) of 193 (for bovine chymotrypsin), compared with k_0 value for BTEE (used in the present study) of 57 (for porcine chymotrypsin) (Folk & Schirmer, 1965).

Although binding of chymotrypsin to the human intestinal mucosa has been noted (Goldberg *et al.* 1969), this phenomenon does not appear to have occurred to a large extent in the present study since the activities measured were at least as high as those of Partridge *et al.* (1982), in pigs with cannulas in the pancreatic duct.

The similarity of the results in the duodenum and jejunum indicated that there was little evidence of extensive loss of enzymic activity in the first part of the small intestine. By the time the ileum was reached much of the activity had been lost but the activity at this site was considerably greater in proportion to the amount of substrate (expressed as total amino acids) than in the duodenum and jejunum for diets BWF and SSG, though not for diet SSC, the protein of which was virtually completely digested by this site (Low, 1979*b*).

Trypsin. As with chymotrypsin the pattern of hourly activity of trypsin in certain instances indicated a response to feeding, and continuous activity during 24 h (but with more activity by day than by night); these findings accord with those of Corring *et al.* (1972).

The mean total activity in the duodenal digesta was similar to that found in pancreatic juice of pigs given diet BWF (Partridge *et al.* 1982) but the amount in the jejunum was greater than that found in pancreatic juice, perhaps because different pigs were used for duodenal and jejunal digesta collections. These apparent differences may have little meaning because of the variability of the values. Values for duodenal digesta, jejunal digesta and pancreatic juice were all similar, however, for pigs given diet SSC. The trypsin activities recorded in this study were approximately twice those given by Corring *et al.* (1972), after taking into account the fact that the latter authors used benzoyl arginine ethyl ester as substrate which has a k_0 value of 22, whereas the k_0 value for TAME is 147 (for bovine trypsin) (Cunningham, 1965). This difference is probably due to the fact that the pigs used in the present study were given twice as much diet as those of Corring *et al.* (1972).

The values for calculated weights of chymotrypsin : trypsin in digesta were higher for each diet in the ileum than in the duodenum or jejunum, suggesting that chymotrypsin was more resistant to proteolysis or inactivation than trypsin. In spite of the uncertainties of the results because of variability it may be noted that the values for diets BWF and SSC in the duodenum are similar to those measured by Partridge *et al.* (1982) in pancreatic juice of pigs fed these diets, in pancreatic juice of pigs collected by Corring *et al.* (1972) and in human duodenal digesta (Goldberg & Wormsley, 1970). The value in the ileum tended to be lower in pigs than in humans, where a value of 1·5 has been recorded (Goldberg & Wormsley, 1970). Deviations from this value are regarded as a sign of disease in humans.

The amount and nature of the endogenous proteins secreted into the gut have been the subject of much research. In the instance of the 50 kg pig it has been estimated that 40–50 g endogenous protein is present in the duodenal digesta during 24 h periods (Zebrowska & Buraczewska, 1972). The results of the present study suggest that pepsin, chymotrypsin and trypsin alone may make up 15–20% of this protein. It is likely that the other pancreatic enzymes may account for another 15–20% of this protein, assuming that the pancreas of 50 kg pigs secretes approximately 8 g protein in 24 h (Partridge *et al.* 1982) and that all the protein is enzymic.

The potential hydrolytic capacity of the gut proteases and peptidases has sometimes been considered to be a factor which limits the digestibility of dietary proteins in pigs. Although it is not valid to carry over values for enzymic activity on esters to the peptide linkages of intact proteins, it appears that the amounts of proteases secreted are far larger than those necessary to hydrolyse all susceptible links in the dietary protein. Although the conditions in the gut are not optimal, because of the presence of enzyme inhibitors, peptide bonds between amino acids which are not readily susceptible to hydrolysis, degradation of the proteases by other proteases etc., it seems possible that production may considerably exceed the amounts needed for complete digestion of dietary proteins. This apparent over-production may, however, be required in part for the digestion of endogenous proteins the amount of

which may be equal to at least half of the daily intake of protein from the diet (Buraczewska, 1980). The high amount of variation in the amounts of enzyme secreted may also indicate that the rates of synthesis are not closely controlled. It is notable that in spite of this high amount of variability, the variation in the apparent digestibility of N measured in the terminal ileum or faeces has a low variability (Low, 1979*a*). Further studies are needed to answer the question of whether the digestion of dietary proteins is limited by the amounts of proteases secreted by growing pigs.

At the outset of this study it was expected that the widely different types of dietary protein would result in differences in the amounts of proteases secreted. However, the very high amount of variation found, possibly heightened by the difficulties involved in large animal experimentation, have masked any differences that might have existed. Even so, the observation that similar variability was found when pancreatic proteases were measured in pancreatic juice before it entered the duodenum suggests that this variation is a fact of life.

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