Artificial rearing of pigs

2.* The time course of milk protein digestion and proteolytic enzyme secretion in the 28-day-old pig

By R. BRAUDE, M. J. NEWPORT AND J. W. G. PORTER

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

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1. The time course of digestion of milk protein was studied in the 28-d-old pig given a test meal of homogenized cow's milk after a preliminary starvation period.

2. The milk was found to clot in the stomach 15–30 min after the meal. The soluble or 'whey' fraction of the stomach contents rapidly passed into the small intestine. Most of the clotted digesta had also left the stomach 2 h after the meal.

3. The distribution of digesta was studied in six equal segments of the small intestine. In general, there were no significant increases in the amount of intestinal contents at any time after the meal when compared with those in starved pigs, suggesting that digestion of milk at this age is a very efficient process.

4. Fractionation of the soluble digesta from the stomach and small intestine in Sephadex G-25 indicated that relatively little proteolysis occurred in the stomach, but in the small intestine digestion proceeded rapidly, producing a considerable increase in free amino acids in the mid-region.

5. The level of proteolytic enzyme activity in the stomach wall was elevated at 15 min after the meal, but thereafter returned rapidly to the prefeeding levels. Increasing the level of feeding increased the enzyme activity of the digesta and stomach wall. The enzyme activity appeared to be mainly adsorbed by the stomach clot.

6. The proteolytic enzyme activity in the pancreas was unaffected by the meal. However, the activity in the contents of the small intestine increased after the meal, reaching a maximum value at 45 min. Some accumulation of enzymes was found in the lower part of the small intestine, except in the region of the distal ileum where a marked decline in enzyme activity occurred. Increasing the level of feeding increased the proteolytic enzyme activity in the contents of the small intestine.

7. The soluble marker polyethylene glycol was not entirely satisfactory as an indicator of the rate of passage of digesta. The concentration of the marker was found to be greater in the soluble stomach fraction than in the clot shortly after the milk had been ingested. The transit time of the marker from ingestion to the terminal ileum was 2–3 h.

Some aspects of the digestion of milk proteins by baby pigs given whole milk either twice daily or at hourly intervals were described by Braude, Mitchell, Newport & Porter (1970). The extent of protein digestion was studied in 28-d-old pigs killed either 1 or 2 h after feeding. The clotting of the diet in the stomach appeared to regulate the rate of emptying of digesta into the small intestine so that a high efficiency of absorption of nutrients was maintained. Little digestion of protein was found in the stomach, whereas proteolysis in the small intestine proceeded rapidly. These studies have now been extended by investigating the time course of digestion of a single test meal by pigs 28–35 d old and by including a study of the proteolytic enzyme activity in the digesta, stomach and pancreas.

Pigs were slaughtered at intervals from 0·25 to 3 h after a meal and the amounts and nature of the nitrogenous compounds were determined in the contents of the stomach

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and small intestine. The pigs used in these studies had been artificially reared from 2 d old in a growth study carried out to assess the nutritive value of cow's milk treated by an ion-exchange procedure for the removal of cationic fission products (Braude, Glascock, Newport & Porter, 1969).

EXPERIMENTAL

Animals and management

Pigs were weaned at 36–48 h of age and allowed access *ad lib.* to a diet of homogenized cow's milk. The management of the animals was as described by Braude *et al.* (1969). The majority of the pigs were obtained from the Institute's herd of Large White sows. A few Large White or Large White × Landrace pigs were obtained from a neighbouring farm.

Experimental design

The pigs used ranged in weight from 8·8 to 13·0 kg; animals were killed 0·25, 0·5, 0·75, 1·0, 1·5, 2·0 and 3·0 h after a test meal. Between five and eight pigs were killed at each time interval and care was taken to ensure similar mean weights for each group.

The pigs were starved for 3 h and then offered 150 ml of homogenized cow's milk containing 1% (w/v) of the soluble marker polyethylene glycol (mol.wt 4000). The meal contained 18·3 g dry matter, 765 mg total N and 596 mg casein N. Each pig was watched to ensure that all the feed was consumed. Most of the pigs drank the milk in 30 s; after 2 min the feed was removed and unless at least 130 ml had been consumed the pigs were returned to unrestricted feeding for a further 24 h, and the procedure was repeated. However, this was necessary for only five out of the forty-six pigs used.

Procedure at slaughter

The pigs were killed by an intracardiac injection of 10 ml of an aqueous solution containing 30 mg sodium pentabarbitone/ml. After opening the body cavity, the stomach was ligated with thread just below the pyloric sphincter and also at the base of the oesophagus. The pancreas was then removed. The stomach was removed and weighed; its contents were emptied into a beaker and the pH value determined immediately with a glass electrode. The stomach contents were centrifuged at 850 *g* for 15 min at 5°. The soluble or 'whey' fraction was decanted off; the residue was termed the insoluble fraction.

The small intestine was freed from the mesentery and divided into six or more segments each about 120 cm in length. Each segment was washed out with 0·9% saline warmed to 37 °. The contents and saline washings from each segment were centrifuged at 1350 *g* for 20 min at 5°. The soluble fraction was decanted off; the residue was termed the insoluble fraction. In many pigs, seven or eight segments of the small intestine were obtained. The results from these animals were recalculated in terms of six segments/pig (assuming an even distribution of digesta within each segment) to enable a ready comparison to be made between animals.

All samples of digesta and organs were kept at −20° until analysed.

Analytical methods

Dry matter

Samples were dried overnight (17 h) in aluminium dishes at 100–105°. This procedure was found to give constant dry weights.

Total N

Samples were digested with 2 ml concentrated sulphuric acid (AR) and 1·20 g potassium sulphate (AR), using 0·05 g mercuric oxide as catalyst.

Each digest was diluted with distilled water to give a solution containing 1–15 mg N/100 ml. The N concentration was estimated with a Technicon AutoAnalyzer (Technicon Instruments Co. Ltd, Chertsey) using alkaline phenate. The procedure given in the Technicon Methodology sheet N-3b was followed, except that 1% (w/v) ethylenediamine tetraacetic acid (AR) was added to the sodium hydroxide solution and 4% (w/v) sulphuric acid was used as a wash between samples.

Non-protein N

Trichloroacetic acid (TCA) solution (100% w/v) was added to the sample to give a final TCA concentration of 10%. The mixture was left at 5° overnight, and the protein precipitate separated by centrifuging at 1350 ***g*** for 30 min. The N content of the supernatant fraction was determined.

Casein N

The procedure described by Rowland (1938) was followed.

Fractionation of protein digestion products by Sephadex-gel filtration

A portion of the soluble fraction of the small intestinal contents and saline washings was freeze-dried. The freeze-dried material was redissolved in 25 ml 0·02 M-sodium phosphate buffer at pH 7·6 and, as a small amount remained insoluble, it was centrifuged at 1200 ***g*** for 10 min; 5 ml of the supernatant fraction containing 2–5 mg N were applied to the Sephadex column. The soluble stomach contents were prepared for analysis in an identical manner, except that the buffer also contained 0·1 M-sodium chloride.

The separation column was prepared with Sephadex G-25 (fine bead form) (Pharmacia, Uppsala, Sweden). The column was 60 cm in length and had a diameter of 2·5 cm. The preparation and calibration of the column was carried out as described by Ford (1965). The samples were eluted from the column in 10 ml fractions using 0·02 M-sodium phosphate buffer at pH 7·6 containing 0·1 M-sodium chloride. Soluble proteins and large peptides were eluted in Fractions 10–16, small peptides in Fractions 17–23 and amino acids in subsequent fractions.

A sample (1 ml) of each fraction was hydrolysed by heating with 2 ml 6 N-HCl for 3 h at 120° in a steam autoclave and, after cooling, 2·4 ml 4 N-sodium hydroxide and 1 ml 4 N-sodium acetate buffer solution at pH 5·5 were added to each sample before making up with water to 10 ml. A portion (1 ml) was taken for the estimation of α-amino N, by reaction with the modified ninhydrin reagent of Moore & Stein (1954).

A standard curve was prepared with graded concentrations of leucine, and the results are expressed in terms of 'leucine equivalent'.

Proteolytic enzyme activities

Preparation of casein for substrates. Proteolytic enzyme activity was estimated by the liberation of tyrosine residues from a solution of casein. A brief account of this method has already been published (Henschel, Hill & Porter, 1961). The substrates were prepared from washed casein precipitated at pH 4·6 from whole milk with HCl.

Activities in the stomach wall and contents. The substrate was prepared by slowly blending the casein obtained from 1 l milk with approximately 500 ml of water containing 21·01 g citric acid monohydrate (AR) in an Ato Mix Blender (Measuring and Scientific Equipment Ltd, London). Further water was added to the solution and its pH value adjusted to 1·8 by the dropwise addition of 0·5 N-HCl to give a final volume of 1 l.

The entire stomach walls were cut into several pieces and homogenized in an Ato Mix Blender with 9 volumes of cold 0·01 N-HCl at pH 2. The homogenate was diluted a further 1:20 with 0·01 N-HCl. The stomach contents were homogenized in 0·01 N-HCl to give an appropriate dilution. To 1 ml of homogenate were added 1 ml 0·01 N-HCl and 3 ml of the casein substrate. The mixture was incubated at 37° for 1 h, when the reaction was stopped by the addition of 10 ml of 5% (w/v) TCA.

A blank determination was carried out for each sample: 1 ml 0·01 N-HCl and 10 ml 5% (w/v) TCA were added to 1 ml of homogenate, followed by 3 ml of the casein substrate, and the mixture was incubated at 37° for 1 h. The extinctions of the TCA filtrates were measured at 277 nm; the difference in extinction between the assay and the blank determinations was proportional to the enzyme activity of the homogenate. The results were calculated with reference to purified pepsin (Koch–Light Laboratories Ltd, Colnbrook), 1 mg of which liberated 0·017 m-equiv. of tyrosine residues per h when incubated with the casein substrate at 37°.

Activities in the pancreas and contents of the small intestine. The substrate was prepared by dissolving the casein obtained from 1 l of milk in 0·05 M-tris buffer at pH 7 containing 2% (w/v) of ethylenediamine tetraacetic acid. Further buffer was added and the pH value of the substrate adjusted to 7·5 with approximately N-sodium hydroxide to give a final volume of 1 l.

The pancreases were homogenized in 0·01 M-tris buffer at pH 7 containing 0·33% (w/v) calcium chloride, and diluted with the buffer to a final concentration of 1:5000. The activity in the contents of the small intestine was estimated in 1 ml of the saline washings without further dilution. In each assay, a blank determination was made on 1 ml of homogenate (or small intestinal contents) which had been boiled for 30 s. Enterokinase (Koch–Light Laboratories Ltd, Colnbrook) was dissolved in 0·01 M-tris buffer at pH 7 containing 0·33% (w/v) calcium chloride to give a concentration of 0·5 mg protein/ml; 1 ml of this solution was added to both the blank and assay samples, and the mixtures were incubated at 37° for 30 min. Three ml of the casein substrate were added, followed by the addition of 10 ml 5% (w/v) TCA to the mixtures for determination of the blank. All samples were then incubated at 37° and,

after 1 h, 10 ml 5 % (w/v) TCA were added to the assay samples. The optical densities of the TCA filtrates were measured at 277 nm, and the difference in optical density between the assay and the blank determinations was proportional to the enzyme activity of the sample. The results were calculated with reference to purified trypsin (Koch–Light Laboratories Ltd, Colnbrook), 1 mg of which liberated 0·022 m-equiv. of tyrosine residues per h when incubated with the casein substrate at 37°.

Polyethylene glycol (PEG)

PEG was analysed by the method of Smith (1958) on portions of whole uncentrifuged stomach contents and the soluble fractions of the washings from the small intestine.

RESULTS AND DISCUSSION

Stomach emptying

The amount and composition of the digesta present in the stomach at different times after a test meal are presented in Table 1. The appearance of the stomach contents from pigs killed 0·25 h after the test meal indicated that clot formation was incomplete at this time, but was complete in the stomachs of pigs killed 0·5 h after feeding. Washburn & Jones (1916) found that milk was clotted 10 min after feeding in the stomach of pigs 27–35 d of age. Under normal conditions, digesta are continuously present in the stomach of pigs of this age and are probably incorporated into the clot formed from milk consumed at subsequent meals (Braude *et al.* 1970). This view is supported from experiments with infant rats, where a regular layering pattern is found in the stomach contents, and after starvation this pattern takes many hours to re-establish (Platt, 1961).

With both the dry matter and N, the patterns of emptying from the stomach were similar for the clot and the soluble fraction. Stomach emptying proceeded rapidly, following the complete clotting of the milk, and was nearly complete 2 h after the meal. The rate of stomach emptying found in these experiments agreed well with results of previous studies carried out on young pigs (Kvasnitskii & Bakeeva, 1940; Kvasnitskii, 1951; Padalikova, 1964). The rate of stomach emptying of infant rats when continuously sucking their dam is a slower process. The amount of stomach clot and its appearance did not change during the 1st h after feeding, and 7 h had elapsed before most of the curd had left the stomach (Naismith, Mittwoch & Platt, 1969). The soluble fraction of the stomach contents of the pigs was found to drain rapidly from the stomach, and had nearly completely disappeared within 0·75 h after the meal (Table 1). This is in good agreement with the results of Guminski & Naismith (1959) who found that most of the soluble fraction left the stomach of the infant rat in 30–40 min. They also reported that 74 % of the casein in the milk was retained in the stomach clot, while the results presented in Table 1 showed that retention of casein in the clot was complete in the 28-d-old pig.

Stomach emptying has been reported to be exponentially related to time for milk-fed fistulated calves (Mylrea, 1966) and the results presented here broadly follow an exponential relationship.

Table 1. *Composition and pH of digesta in the stomach of pigs between 28 and 35 d old at different times after a meal of 150 ml homogenized cow's milk*

(Mean values with their standard errors)

Time after feeding (h)	No. of pigs	Dry matter (g)		N (mg)		pH	Concentration in soluble fraction			
									NPN	
		Insoluble	Soluble	Insoluble	Soluble		Dry matter (mg/ml)	N (mg/ml)	mg/ml	As % of total soluble N
0*	8	1·38 ± 0·78	0·20 ± 0·12	116 ± 83	8·4 ± 5·3	4·0 ± 0·6	25·2 ± 5·2	1·13 ± 0·27	0·66 ± 0·38	58·4 ± 13·2
0·25	6	12·34 ± 2·24	4·69 ± 0·49	588 ± 92	134·7 ± 26·0	5·4 ± 0·1	63·1 ± 1·9	1·73 ± 0·31	0·35 ± 0·07	20·2 ± 3·1
0·5	5	12·58 ± 2·13	0·89 ± 0·29	600 ± 101	33·1 ± 9·8	4·4 ± 0·2	81·7 ± 12·5	3·32 ± 0·36	0·75 ± 0·15	22·6 ± 3·9
0·75	5	4·15 ± 0·99	0·22 ± 0·05	231 ± 52	9·4 ± 2·4	3·5 ± 0·3	43·6 ± 10·6	1·94 ± 0·58	0·67 ± 0·23	34·5 ± 7·0
1·0	5	7·31 ± 1·59	0·29 ± 0·11	297 ± 76	19·1 ± 7·1	2·4 ± 0·3	42·4 ± 5·1	2·69 ± 0·25	0·91 ± 0·17	33·8 ± 6·9
1·5	6	5·09 ± 1·72	0·39 ± 0·09	251 ± 84	20·1 ± 1·5	3·0 ± 0·6	38·5 ± 3·4	1·72 ± 0·25	0·68 ± 0·12	39·5 ± 8·9
2·0	6	3·35 ± 1·07	0·17 ± 0·09	146 ± 53	8·1 ± 4·3	3·3 ± 0·4	33·1 ± 6·8	1·77 ± 0·45	1·23 ± 0·28	69·5 ± 4·1
3·0	5	0·20 ± 0·07	0·17 ± 0·10	13 ± 5	13·5 ± 9·1	6·7 ± 0·1	44·7 ± 1·0	1·88 ± 0·78	—†	—

NPN, non-protein N. * Killed after a fast of 3 h. † Insufficient material available.

Digestion in the stomach

Initially, the pH of the stomach contents decreased linearly with time, reaching a minimum value of 2·4 at 1 h after the meal. This is near to the optimum pH for pepsin activity (1·8), and should permit rapid proteolysis. At intervals longer than 1 h after the meal, some regurgitation of intestinal contents occurred, as demonstrated by the presence of bile pigments in the stomach contents, and the pH value gradually increased with time. The maintenance of a low pH value in the stomach contents resulted in some proteolysis, as indicated by an increase in the NPN relative to the total soluble N. Naismith *et al.* (1969) concluded that little protein hydrolysis occurred in the stomach of infant rats, and in their experiments the amount of NPN as a percentage of the total N increased from 11·2 immediately after feeding to 20·3 after 3 h. Walker (1959) found a mean pH value of 3·5 in the stomach contents of pigs killed 2 h after sucking, compared with a value of 3·3 found in the present experiment. Maner, Pond, Loosli & Lowrey (1962), using 28-d-old pigs fitted with gastric fistulas, found a pH value of 2 in the stomach contents 2 h after giving a meal containing casein and the decline in pH was even more rapid in 56-d-old pigs. Noakes, Cranwell & Hill (1968) reported that gastric secretion in pigs between 42 and 112 d of age reached a maximum 1·5–2·5 h after feeding, and declined to prefeeding levels after 3·5–5 h. The juice had a high acidity and a low proteolytic enzyme activity, although the total proteolytic enzyme secretion increased after feeding.

Amount and composition of digesta in the small intestine

The amounts of digesta present in each of the six segments and in the entire small intestine are shown in Table 2. Little difference was apparent in the distribution or total amounts of digesta at any time after the meal.

There was no significant increase in the total amount of soluble N at any time after the meal, indicating that the milk protein was rapidly digested and absorbed. Similarly, little accumulation of soluble N was found in the intestinal contents of rats given a diet containing casein (Peraino, Rogers, Yoshida, Chen & Harper, 1959; Chen, Rogers & Harper, 1962; Rogers & Harper, 1964; Harper, 1965). Thus, it may reasonably be supposed that when a diet containing readily absorbed protein is given, the soluble N in the intestinal contents is mainly of endogenous origin.

Fractionation of protein digestion products by Sephadex-gel filtration

The relative amounts of proteins, peptides and amino acids obtained after fractionation of the soluble digesta in Sephadex G-25 are shown in Table 3.

Examples of the patterns of the digestion products in the stomach and small intestine are shown in Fig. 1. It is evident that large amounts of proteins and peptides, relative to amino acids, were present in the soluble stomach fraction, whereas in the small intestine rapid hydrolysis of the proteins and peptides led to some accumulation of amino acids. In the small intestine, the increase in the amino acid fraction occurred mainly in the middle region. In agreement with this, Clark, Hays, McCall & Speer

Table 2. *Amount and composition of digesta in the small intestine of pigs between 28 and 35 d old at different times after a meal of 150 ml homogenized cow's milk*

Time after feeding (h)	No. of pigs	Insoluble N (mg)								Soluble N (mg)							
		Amount (mg) in segment no.						Total	LSD* between segments	Amount (mg) in segment no.						Total	LSD* between segments
		1	2	3	4	5	6			1	2	3	4	5	6		
0†	8	3·7	6·1	7·8	7·7	2·8	3·9	32·0	9·9	42	64	77	67	55	60	365	88
0·25	6	3·5	3·9	4·3	6·3	5·7	4·6	28·3	4·5	27	40	54	92	66	39	318	39
0·5	5	3·7	1·6	2·1	3·3	3·7	4·6	19·0	2·2	42	18	44	80	75	61	320	35
0·75	5	3·9	5·4	6·3	8·3	4·3	5·0	33·2	6·4	40	57	77	108	88	52	422	47
1·0	5	7·2	9·2	3·6	2·3	2·3	2·9	27·5	4·7	77	121	76	62	66	39	441	74
1·5	6	3·1	3·3	2·3	3·2	2·6	2·4	16·9	2·1	23	26	45	70	62	42	268	24
2·0	6	12·6	13·6	6·1	1·8	2·7	3·7	40·5	10·1	106	86	81	22	64	55	414	63
3·0	5	3·7	6·1	7·8	7·7	2·8	3·9	32·0	9·3	22	27	24	38	39	59	209	21
LSD* between times		7·2	8·7	5·5	6·9	3·1	3·9	12·8	—	66	68	54	52	42	38	244	—
		Insoluble dry matter (mg)								Soluble dry matter (g)							
0†	8	133	197	293	214	115	215	1167	215	0·31	0·62	0·59	0·54	0·68	0·75	3·49	0·52
0·25	6	137	174	212	190	258	153	1124	140	0·23	0·37	0·46	1·07	0·61	0·39	3·13	0·34
0·5	5	188	47	85	188	168	209	885	122	0·59	0·31	0·83	1·15	1·18	0·80	4·86	0·48
0·75	5	177	226	301	381	196	189	1470	240	0·46	0·37	0·78	1·41	1·35	0·60	4·97	0·50
1·0	5	393	402	190	123	117	99	1324	270	0·70	1·24	0·97	0·99	1·14	0·60	5·64	0·63
1·5	6	86	108	129	196	134	115	768	100	0·16	0·20	0·69	1·20	1·11	0·49	3·85	0·44
2·0	6	335	393	312	94	142	181	1457	200	0·80	0·86	0·73	0·33	1·46	0·70	4·88	0·54
3·0	5	65	102	81	117	167	403	935	180	0·13	0·13	0·22	0·44	0·81	1·10	2·83	0·42
LSD* between times		226	266	205	250	128	226	780	—	0·54	0·58	0·59	0·45	0·57	0·51	2·32	—

Segment 1, proximal duodenum; segment 6, distal ileum.

* Least significant difference for $P < 0·05$. The between-times LSD was calculated from the harmonic mean.

† Killed after a fast of 3 h.

(1963) reported higher levels of essential amino acids in the jejunum than in both the duodenum and the ileum of baby pigs given a diet containing casein.

When rats were given a meal of casein, and the soluble N in the small intestine was fractionated in Sephadex G-25, the peptide fraction was usually greater than the protein fraction and sometimes nearly as large as the amino acid fraction (Zebrowska, 1968). Only a small proportion of the soluble N in the small intestine of the baby pig was found to be peptides. It is possible that in the baby pig the rate of hydrolysis of peptides to amino acids within the small intestine temporarily exceeds the rate of amino acid absorption.

Table 3. *Relative and total amounts, in pigs, of α-amino nitrogen in the 'protein', 'peptide' and 'amino acid' fractions of the soluble digesta after separation in Sephadex G-25*

		Total leucine equivalent (mg)			
Time after feeding (h)	Site*	Protein fraction	Peptide fraction	Amino acid fraction	Leucine equivalent (mg/mg soluble N)
0†	1	35	29	24	10·50
	2	431	67	401	8·48
	3	135	114	927	8·18
	4	144	89	680	7·93
0·25	1	672	159	172	7·43
	2	182	91	330	9·01
	3	155	0	1389	10·57
	4	172	59	640	8·28
1·5	1	67	47	34	7·35
	2	91	45	345	9·82
	3	294	56	795	9·96
	4	281	47	640	9·30

The samples from all pigs slaughtered at each time interval were pooled for analysis.
* 1, stomach; 2, proximal small intestine; 3, middle of small intestine; 4, distal small intestine.
† Killed after a fast of 3 h.

Proteolytic enzyme activity

The proteolytic enzyme activity in the stomach wall, pancreas and digesta from 28-d-old pigs fed at various levels, and at either hourly intervals or twice daily, is given in Tables 4 and 5. The levels of feeding used, the performance of the pigs and the amount and composition of the digesta in various regions of the alimentary tract have been described previously (Braude *et al.* 1970). Scale A represents a low level of feeding gradually increasing to the highest level, scale D. The proteolytic enzyme activity/g digesta in the stomach was higher in pigs fed at hourly intervals, although the large variation resulted in mainly non-significant differences (Table 4). The total enzyme activity was increased at the higher levels of feeding, but was not influenced by the frequency of feeding. The enzyme activity/g stomach wall was not influenced by either frequency or level of feeding. However, the total activity of the stomach wall was higher at the higher levels of feeding.

The proteolytic enzyme activity of the pancreas was unaffected by either level or frequency of feeding (Table 5). However, at high levels of feeding, the contents of the small intestine were found to have a higher enzyme activity than in pigs fed at lower levels.

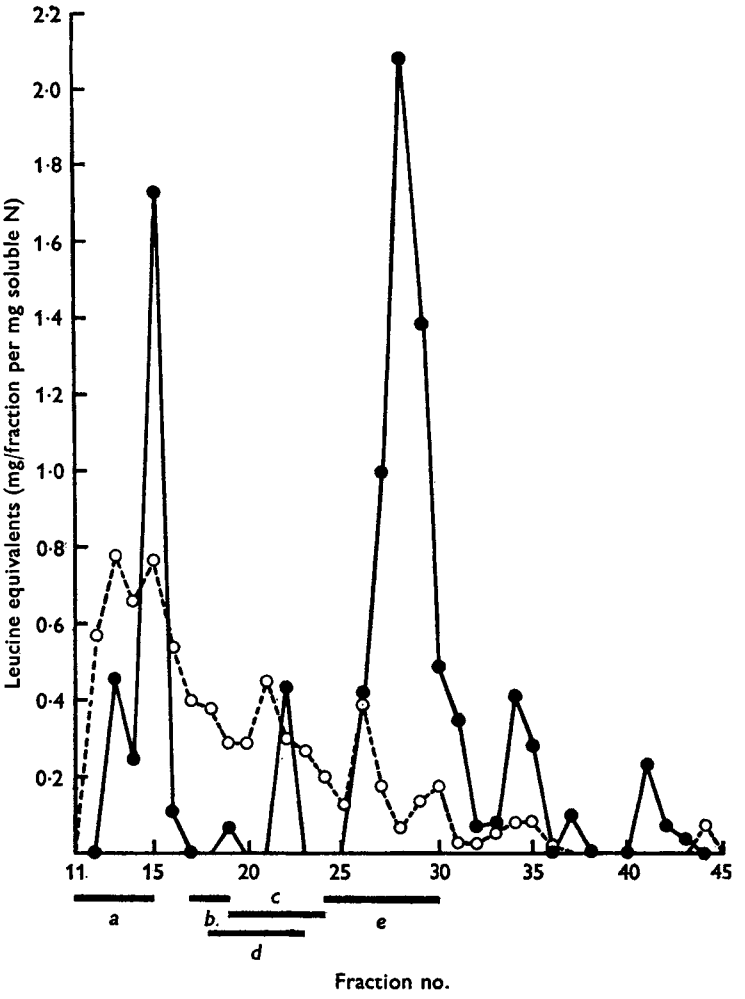

Fig. 1. Fractionation in Sephadex G-25 of the soluble nitrogen contained in the digesta from the middle region of the small intestine (●—●) and the stomach contents (○- - -○) of pigs 28–35 d old, 1·5 h after a feed of homogenized cow's milk. The bars under the abscissa depict the position of the 'markers' in the effluent: *a*, cytochrome-*c*, mol. wt 12400; *b*, insulin B chain, mol. wt 3483; *c*, polymixin B sulphate, mol. wt 1447; *d*, bacitracin A, mol. wt 1470; *e*, a mixture of eighteen amino acids.

When pigs were killed at different intervals after a single test meal the proteolytic enzyme activity in both the wall and contents of the stomach was higher at 0·25 h after the meal than in the starved pigs (Table 6), indicating that secretion by the tissues of the stomach wall had been stimulated by the meal. After this initial increase the amount of the enzyme in the stomach wall rapidly returned to prefeeding levels.

Using calves with abomasal pouches and abomasal fistulas, Ash (1964) also reported increased proteolytic activity in the abomasum when milk was given, followed by a rapid decline to below prefeeding levels.

The proteolytic enzyme activities in the pancreases of the pigs are given in Table 7 and those in the contents of the small intestine in Table 8. Neither the concentration

Table 4. *Effect of frequency and level of feeding on the proteolytic enzyme activity of the stomach contents and of the stomach wall in 28-d-old pigs*

Treatment no. ...		1	2	3	SEM and differences significant at the 5 % level			
Frequency of feeding ...		Hourly	Twice daily					
Time after feeding (h) ...		1	1	2	Within-scales		Between-scales*	
	Feeding scale†							
Enzyme activity in contents: μg/g wet digesta	A	78	22	54	38	NS	23	NS
	B	125	59	55	14	1 > 2, 3		
	C	148	46	—	31	NS		
	D	97	—	—	3	—		
Total (mg)	A	2·9	5·7	4·6	1·9	NS	1·8	C > A, B (2) C, D > A (1)
	B	5·9	5·9	4·5	1·0	NS		
	C	14·4	13·5	—	3·4	NS		
	D	10·4	—	—	0·5	—		
Enzyme activity in stomach wall: mg/g wet tissue	A	2·57	2·44	2·57	0·26	NS	0·27	C > A, B (1)
	B	1·98	2·53	2·06	0·27	NS		
	C	3·11	3·20	—	0·38	NS		
	D	2·68	—	—	0·36	—		
Total (mg)	A	95	113	105	14	NS	13	C, D > A, B (1) C > A, B (2)
	B	90	120	104	13	NS		
	C	167	179	—	16	NS		
	D	156	—	—	19	—		

The results are mean values for between five and fourteen pigs. The standard errors were calculated from the harmonic mean. The between-scales (vertical comparisons) SEM was calculated from the combined residual errors for each scale of feeding.

NS, not significant. * Treatment numbers in parentheses. † See Braude *et al.* (1970).

Table 5. *Effect of frequency and level of feeding on the proteolytic enzyme activity of the pancreas and contents of the small intestine of 28-d-old pigs*

Treatment no.		1	2	3	SEM and differences significant at the 5 % level			
Frequency of feeding		Hourly	Twice daily					
Time after feeding (h)		1	1	2	Within-scales		Between-scales*	
Feeding scale†								
Proteolytic enzyme activity of pancreas: mg/g wet tissue	B	74	82	78	11·8	NS	8·0	NS
	C	53	56	—	2·1	NS		
	D	49	—	—	1·6	—		
Total (mg)	B	910	1000	940	164	NS	113	NS
	C	690	720	—	59	NS		
	D	760	—	—	41	—		
Proteolytic enzyme activity in the small intestine contents (mg)	B	96	105	126	5	3 > 1, 2	7	C, D > B (1) C > B (2)
	C	190	196	—	14	NS		
	D	130	—	—	8	—		

The results are mean values for between five and twelve pigs. The standard errors were calculated from the harmonic mean. The between-scales (vertical comparisons) SEM was calculated from the combined residual errors for each experiment.

NS, not significant. * Treatment numbers in parentheses. † See Braude *et al.* (1970).

nor the total activities in the pancreas were affected by feeding, and great variability was observed between individual pigs. In the contents of the small intestine the levels of activity increased distally and the highest levels were found in the fourth and fifth segments; a lower level of activity was found in the terminal segment and may have been

Table 6. *Proteolytic enzyme activity in the stomach contents and stomach wall of pigs between 28 and 35 d old at different times after a meal of 150 ml homogenized cow's milk*

		Stomach contents				Stomach wall	
		Insoluble		Soluble			
Time after feeding (h)	No. of pigs	μg/g	Total (mg)	μg/g	Total (mg)	mg/g wet tissue	Total (mg)
0*	8	211	2·4	56	0·4	1·36	74
0·25	6	186	13·4	58	4·2	3·71	228
0·5	5	139	6·9	33	0·2	1·54	90
0·75	4	191	4·6	52	0·3	2·12	123
1·0	5	187	4·0	22	0·2	1·76	97
1·5	6	180	3·1	18	0·2	2·12	114
2·0	6	171	2·9	42	0·9	1·76	103
3·0	5	156	0·6	16	0·3	1·08	54
SEM†	—	57	2·8	14	0·15	0·64	41
Differences significant at the 5 % level		NS	0·25 > all other times	0, 0·25 > 1·0, 1·5, 3·0; 0·75 > 3·0	0·25 > all other times	0·25 > 0, 0·5, 1·0, 2·0, 3·0	

NS, not significant. * Killed after a fast of 3 h. † Calculated from the harmonic mean.

Table 7. *Proteolytic enzyme activity in the pancreas of pigs between 28 and 35 d old at different times after a meal of 150 ml homogenized cow's milk*

		Pancreas	
Time after feeding (h)	No. of pigs	mg/g wet tissue	Total (g)
0*	3	90	1·59
0·25	6	80	2·19
0·5	5	63	1·21
0·75	3	78	1·56
1·0	4	54	1·14
1·5	6	104	1·63
2·0	5	96	1·99
3·0	5	105	1·65
SEM†	—	28	0·80

The differences were not significant at the 5 % level.
* Killed after a fast of 3 h. † Calculated from the harmonic mean.

due to autolysis. The high levels of proteolytic enzyme activity found in the distal region of the small intestine are in agreement with studies carried out on rats by Pelot & Grossman (1962) and Lepkovsky, Furuta, Ozone, Koike & Wagner (1966).

The proteolytic enzyme activity relative to soluble N content was also greater at 0·75 h and 1 h after the meal. At each time interval, the relative enzyme activity increased along the intestine as more of the dietary N was absorbed, followed by a

Table 8. *Proteolytic enzyme activity, expressed as mg (A) or mg/mg soluble nitrogen (B), in the digesta from segments nos.* 1–6 *of the small intestine of pigs between* 28 *and* 35 *d old at different times after a meal of* 150 *ml homogenized cow's milk*

Time after feeding (h)	1		2		3		4		5		6		Mean	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0*	3·6	0·13	12·9	0·22	16·3	0·18	29·0	0·40	33·5	0·60	10·5	0·21	17·6	0·29
0·25	5·4	0·20	15·6	0·39	30·0	0·56	135·0	1·47	35·0	0·53	11·8	0·30	38·8	0·58
0·5	8·6	0·21	9·7	0·54	55·0	1·25	70·7	0·88	21·8	0·29	19·4	0·32	30·9	0·58
0·75	10·5	0·26	14·5	0·25	58·8	0·76	138·7	1·28	180·5	2·06	22·4	0·43	70·9	0·84
1·0	41·7	0·54	138·0	1·14	34·7	0·46	91·8	1·48	33·6	0·51	3·0	0·08	57·1	0·70
1·5	3·0	0·13	4·5	0·17	12·9	0·29	33·6	0·48	21·4	0·35	10·3	0·25	14·3	0·28
2·0	11·8	0·11	7·5	0·09	21·3	0·26	4·5	0·20	36·5	0·57	11·2	0·20	15·5	0·24
3·0	4·5	0·20	5·3	0·20	6·2	0·26	16·0	0·40	96·3	2·47	15·4	0·26	24·0	0·63
Mean	11·1	0·22	26·0	0·38	29·4	0·50	64·9	0·82	57·3	0·92	13·0	0·26	—	—

Analyses were carried out on a pooled sample from all individuals at each time interval. Segment 1, proximal duodenum; segment 6, distal ileum.
* Killed after a fast of 3 h.

decline in the ileum, possibly due to autolysis of the proteolytic enzyme when all the soluble protein from the diet had been digested.

The total enzyme activity in the contents of the small intestine was also greatest 0·75 h after the meal, and at the time when stomach emptying was most rapid. After

Table 9. *Amount of polyethylene glycol (mg) in the stomach contents of pigs between 28 and 35 d old at different times after a meal of 150 ml homogenized cow's milk*

Time after feeding (h)	Clotted digesta	Soluble digesta
0·25	336	647
0·5	199	44
0·75	40	14
1·0	43	16
1·5	31	10
2·0	8	3
3·0	0	1

Each pig received 1500 mg polyethylene glycol in the meal. Analyses were carried out on a pooled sample from all individuals at each time interval.

Table 10. *Distribution of polyethylene glycol as a percentage of the amount ingested in segments nos. 1–6 of the small intestine of pigs between 28 and 35 d old at different times after a meal of 150 ml homogenized cow's milk*

Time after feeding (h)	1	2	3	4	5	6
0·25	—	—	—	—	—	—
0·5	—	10	18	12	—	—
0·75	—	—	15	27	10	—
1·0	—	14	20	18	10	—
1·5	—	—	18	27	22	—
2·0	—	—	—	12	58	—
3·0	—	—	—	10	28	16

The analyses were carried out on a pooled sample from all individuals at each time interval. Segment 1, proximal duodenum; segment 6, distal ileum. Where no values are given, the amount of polyethylene glycol was less than 10% of that ingested.

1·5 h there was a marked decline in enzyme activity. These findings accord with those of Pekas, Thompson & Hays (1966) who showed that the output of proteolytic enzyme activity from the pancreas of a young pig fitted with a pancreatic fistula increased with time after a meal containing casein.

Rate of passage of digesta

The distribution of the soluble marker PEG in the stomach contents is given in Table 9 and in the contents of the segments of the small intestine in Table 10. As the contents of the small intestine were considerably diluted with the saline during washing procedure, the quantity of PEG present in a segment could only be estimated with reasonable accuracy when 10% or more of the total amount given was present in that segment. Thus, the results for the small intestine are only of a semi-quantitative nature. The main body of the marker reached the terminal ileum 2–3 h after feeding, which agrees with the value of 3 h found by Smith (1964) for the milk-fed calf.

It was clearly shown (Table 9) that most of the PEG consumed in the milk given was not taken up into the stomach clot but remained in the whey fraction and rapidly emptied from the stomach. Thus, the observed movement of marker through the small intestine was mainly related to this fraction of the meal, limiting its value for the estimation of the movement and net absorption of N in this type of diet.

These results are also similar to other studies with baby pigs. Kidder, Manners & McCrea (1961) reported large quantities of barium sulphate marker (as indicated by radiography) in the small intestine of baby pigs 40 min after feeding. In a subsequent study, Kidder & Manners (1968) found that some barium sulphate entered the small intestine soon after feeding and all had left the stomach within 1·5–2 h; little difference was found between 7- and 21-d-old pigs.

The absence of measurable amounts of PEG in the proximal regions of the small intestine indicated a rapid flow of digesta in this region. This pattern of flow is likely to be similar to that occurring in the growing pig (Noakes, Hill, Freeman & Annison, 1967) where the rate of flow of digesta is considerably slower in the distal than in the proximal regions of the small intestine.

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