The course of digestion of different food proteins in the rat. Fractionation of the nitrogen in intestinal contents

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1. The course of digestion of casein, α-protein (an isolated soya-bean protein) and of raw and heated soya-bean meal in the rat was examined and compared by analysis of the stomach and small intestine contents 1, 2, 3 and 4 h after giving test meals of the different proteins.

2. After centrifugation, the soluble nitrogenous compounds of the intestinal contents were separated by filtration on Sephadex gel G-25 into protein, peptide and amino acid fractions.

3. The rates of stomach emptying varied widely; they were faster with α-protein than with casein, and both these proteins left the stomach more quickly than did the two soya-bean meals.

4. The intestinal contents of rats given raw soya-bean meal contained considerably larger amounts of insoluble N and dry matter than did those of rats given the heated soya-bean meal; these, in turn, contained considerably more than did those of rats given casein or α-protein.

5. The intestinal contents of rats given α-protein or raw soya-bean meal contained considerably larger amounts of soluble N than did those of rats given casein or the heated soya-bean meal.

6. Fractionation in Sephadex G-25 of the soluble N of the intestinal contents of the rats given casein showed reduction with time in the amount of proteins, peptides and amino acids but the amounts of all these compounds were considerably less than in the corresponding fractions from the gut contents of the rats given α-protein, which was digested much more slowly.

7. Similar fractionations of intestinal contents of rats given raw soya-bean meal showed an unchanging and very high content of undigested protein, probably of endogenous origin, and only small amounts of the peptide and amino acid fractions, whereas after giving the heated meal the initially large protein fraction progressively decreased.

Buraczewski (1966) studied the course of digestion in the rat of casein, α-protein, ADM-assay protein, soya-bean meals and fish meals. He found large differences in the concentrations of nitrogen in the small intestine and correspondingly wide variations in the concentrations of free amino acids in the blood. He discussed these findings in relation to the nutritive quality of the protein, with particular reference to the relationship between the content of free amino acids in the portal blood plasma and the amino acid composition of the test protein.

Ford (1965) and Ford & Salter (1966) studied the digestion of fish meal in vitro. They found marked differences in the pattern of digestion of heated and unheated meals during incubation with proteolytic enzymes. They analysed the digest into three fractions containing 'soluble proteins', 'peptides' and 'free amino acids' by filtration through a calibrated column of Sephadex gel G-25. The amino acid composition of these fractions was determined and discussed in relation to the availabilities of the amino acids in the original test proteins as measured in microbiological and chemical tests. Buraczewski, Buraczewska & Ford (1967) continued this work and used the technique of Sephadex analysis to investigate the course of digestion of

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heated and unheated cod-muscle protein. In experiments with rats they analysed the contents of the stomach and small intestine in relation to the time elapsed since the test meal, and compared the results with those of digestibility tests in vitro. They found a broad similarity between the results obtained in vivo and in vitro. With increasing severity of heating the solubility of the test protein decreased, as did the rate at which it was hydrolysed by pepsin and subsequently by pancreatin and erepsin. They found, unexpectedly, a relatively high concentration of free α-amino nitrogen in the intestinal contents of rats given severely heated protein, and postulated that the accumulated intermediate products of protein digestion might hinder the absorption of amino acids by saturating the sites involved in the transport of the amino acids across the mucosal barrier.

The present paper reports an extension of the work of Buraczewski *et al.* (1967). The course of digestion of casein, α-protein, and of raw and heated soya-bean meals was examined and compared by analysis of the contents of the stomach and small intestine of rats 1, 2, 3 and 4 h after being given a test meal of the different proteins. The gut contents were fractionated in Sephadex gel G-25 as described by Buraczewski *et al.* (1967).

EXPERIMENTAL

Protein sources

Casein (vitamin-low) was obtained from Fisons Pharmaceuticals Ltd, Loughborough, and α-protein from Glidden Co., Chicago, Illinois. Raw and heated commercial defatted soya-bean meals containing 50 % protein were obtained from Central Soya, Decatur, Indiana, and from Central Soya Co., Chemurgy Division, Chicago, USA, respectively.

Tests with rats

Adult male hooded Norwegian rats weighing 300–400 g were used. For some months before use they were maintained on a commercial pelleted diet. About 40 h before being offered the test meal, the rats were put into tubular coprophagy-preventing cages of the type described by Metta, Nash & Johnson (1961). The test materials were given after a fasting period of 18 h in amounts calculated to supply 24·3 mg N/100 g body-weight and were usually consumed within 15 min. At 1, 2, 3 and 4 h after consuming the test meal, the rats were anaesthetized and the gut was exposed. The small intestine was ligated at the pylorus and cannulated just distal to this point. The ileal end was transected at the junction with the caecum, and the contents of the small intestine were washed out with about 70 ml of 0·9 % (w/v) NaCl solution into a small beaker which was then rapidly cooled in ice-water. The stomach was removed and the contents were washed into a small beaker with about 30 ml distilled water.

Preparation of the gut contents for analysis. The contents of the stomach and small intestine were centrifuged for 20 min at 1250 *g* at a temperature below 10°. The supernatant fluid was decanted off. The residue was resuspended in 10 ml water and centrifuged again, and the washings were combined with the original supernatant liquor. The washed residue was transferred to a dish for the determination of dry matter and, subsequently, of 'insoluble N'. The supernatant fluids from the stomach contents were

made up with water to 50 ml, and those from intestinal contents were diluted with saline to 100 ml. Portions were taken for determination of dry matter, and of N by the micro-Kjeldahl method. Portions of the soluble intestinal contents from the individual rats in each group containing equal amounts of N were then pooled. The pooled preparations were freeze-dried and redissolved in 0·02 M-phosphate buffer at pH 7·6.

Fractionations on Sephadex gel

Sephadex gel filtration medium was used, of fine bead form, type G-25, particle size 20–80 μm (Pharmacia, Uppsala, Sweden). The column, of height 60 cm and internal diameter 2·5 cm, prepared and calibrated as described by Ford (1965), had a void volume of 140 ml. Portions (5 ml) of the samples were applied to the column and eluted at a rate of 120 ml/h with 0·02 M-sodium phosphate buffer at pH 7·6 containing 0·1 M-NaCl. Soluble proteins and peptides of molecular weight greater than about 4000 were eluted between 130 and 160 ml, peptides of molecular weight between 4000 and 250 were eluted between 170 and 250 ml, and 'free amino acids' between 260 and 340 ml. Some of the phenylalanine and nearly all the tyrosine emerged between 350 and 450 ml. Tryptophan followed between 540 and 700 ml, but was not collected.

One ml of each fraction was heated in a steam autoclave with 2 ml of 6 N-HCl for 3 h at 120°. To each hydrolysate were added 2·4 ml 4 N-NaOH and 1 ml of 4 N-sodium acetate buffer solution at pH 5·5, and made up to 10 ml with water. A 1 ml portion 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 concentration of leucine, and the results were expressed in terms of 'leucine equivalent'.

RESULTS AND DISCUSSION

Stomach contents

In Table 1 are shown the soluble and insoluble N and dry-matter contents in the stomach of rats at 1, 2, 3 and 4 h after eating the different proteins. In Table 2 the total N and dry-matter contents of the stomach remaining at the different times are expressed as percentages of the amounts given.

It is apparent from Tables 1 and 2 that the rate of stomach emptying was different with the proteins studied. Thus α-protein left the stomach more rapidly than casein, but both these proteins left more quickly than raw and heated soya-bean meals. There was little difference between the rates of stomach emptying of the two soya-bean meals.

Intestinal contents

The soluble and insoluble N and dry-matter contents of the intestinal contents of rats 1, 2, 3 and 4 h after test meals of the different proteins are shown in Table 3.

Casein. The amount of insoluble N in the intestinal contents after the casein meal was very low and there was no appreciable change with time. The soluble N and dry-matter contents reached peaks at 2 h after feeding and then declined. The insoluble dry-matter content showed little change with time.

α-Protein. Again the amount of insoluble N was small, though perhaps a little larger than with casein. Unexpectedly, there were comparatively large accumulations of soluble N and dry matter. At 1 h after feeding the N content was 4·36 mg/100 g body-weight, and the highest concentration, 6·98 mg/100 g, was found at 2 h; and at 3 h the concentration had fallen only to 5·78 mg/100 g, as against 1·96 mg with casein.

Table 1. *Nitrogen and dry-matter contents of the stomach of the rats 1, 2, 3 and 4 h after ingesting different proteins*

(Values are expressed as mg/100 g body-weight and are means, with their standard errors, for six rats)

Diet				Content in stomach			
	Amount fed			Nitrogen		Dry matter	
Protein	Nitrogen	Dry matter	Time after feeding (h)	Insoluble	Soluble	Insoluble	Soluble
Casein	24·3	167	1	9·19±0·73	1·98±0·12	63·5±5·22	17·6±1·62
			2	3·99±0·33	1·92±0·15	26·6±1·98	16·1±1·05
			3	1·29±0·35	1·72±0·23	11·5±2·66	13·9±2·06
			4	0·47±0·19	0·55±0·14	4·20±1·53	4·50±1·03
α-Protein	24·3	170	1	7·69±0·56	2·21±0·27	60·1±6·03	19·6±2·26
			2	3·21±0·34	1·94±0·18	23·9±2·15	12·9±1·10
			3	0·27±0·03	0·67±0·31	3·63±0·77	8·23±2·66
Raw soya-bean meal	24·3	297	1	11·6±0·36	1·84±0·10	132·4±7·40	41·7±1·65
			2	7·87±0·56	1·24±0·15	87·9±6·22	25·2±2·64
			3	5·07±0·27	1·21±0·12	71·3±9·32	21·7±1·58
			4	1·68±0·23	0·90±0·12	27·5±3·53	13·9±2·51
Heated soya-bean meal	24·3	301	1	11·4±0·81	1·32±0·06	139±14·61	32·7±0·80
			2	7·17±0·47	1·09±0·06	74·2±5·62	23·4±1·02
			3	3·45±0·97	0·86±0·07	37·4±9·40	14·0±2·10
			4	1·79±0·41	0·63±0·12	18·4±5·16	7·96±1·38

Table 2. *Nitrogen and dry-matter contents of the stomach contents of the rats, expressed as percentages of the amounts fed**

Diet	Time after feeding (h)	Nitrogen	Dry matter
Casein	1	46	48·5
	2	24·3	25·6
	3	13·4	15·2
	4	4·20	5·21
α-Protein	1	40·9	46·9
	2	21·3	21·6
	3	3·88	6·95
Raw soya-bean meal	1	55·3	57·8
	2	37·5	37·6
	3	25·8	30·9
	4	10·62	13·77
Heated soya-bean meal	1	52·1	57·9
	2	38·2	32·9
	3	17·8	17·3
	4	9·96	8·89

* Amounts fed are given in Table 1.

Raw soya-bean meal. The content of insoluble N was markedly higher with the raw soya-bean meal than with casein and α-protein. The soluble N content was also high, as with α-protein, and declined only slowly from a peak value of 5·50 mg/100 g body-weight at 2 h to 3·74 mg at 4 h.

Table 3. *Nitrogen and dry-matter contents of the small intestine of rats 1, 2, 3 and 4 h after ingesting different proteins*

(Values are expressed as mg/100 g body-weight and are means, with their standard errors, for six rats)

Diet	Time after feeding (h)	Content of small intestine: Nitrogen, Insoluble	Nitrogen, Soluble	Dry matter, Insoluble	Dry matter, Soluble
Casein	1	0·16±0·01	2·82±0·27	3·75±0·48	30·7±3·63
	2	0·11±0·01	3·15±0·16	3·72±0·68	35·2±2·98
	3	0·12±0·01	1·96±0·10	2·47±0·43	22·8±0·65
	4	0·17±0·02	1·72±0·09	4·53±1·10	24·4±2·01
α-Protein	1	0·22±0·03	4·96±0·32	4·69±1·18	62·2±4·65
	2	0·19±0·02	6·98±0·27	3·39±0·34	58·1±2·05
	3	0·23±0·11	5·78±0·41	4·64±0·75	52·6±8·61
Raw soya-bean meal	1	1·01±0·11	5·16±0·32	22·7±2·71	68·7±3·17
	2	1·12±0·17	5·50±0·36	27·8±3·11	88·9±9·46
	3	1·07±0·06	5·07±0·20	31·8±2·21	70·5±4·84
	4	0·85±0·07	3·74±0·39	31·4±2·21	71·6±2·94
Heated soya-bean meal	1	0·69±0·04	3·99±0·15	25·6±1·37	71·9±3·78
	2	0·58±0·07	2·59±0·08	28·8±2·16	66·0±2·45
	3	0·59±0·03	2·73±0·16	37·3±1·80	66·1±4·99
	4	0·52±0·09	2·60±0·16	27·6±3·41	73·7±4·57

The amounts of soluble and insoluble dry matter were comparatively large, no doubt because of the insoluble and poorly digested carbohydrates present in soya-bean meal.

Heated soya-bean meal. With heated soya-bean meal there was no accumulation of soluble N such as occurred with the unheated meal. The highest concentration, 3·99 mg/100 g body-weight, was found at 1 h after feeding; thereafter the level of N declined to 2·6 mg/100 g body-weight at 2 h, and remained at about this level at 3 and 4 h. There was only about half as much insoluble N with the raw meal.

Thus proteins of the same origin, α-protein and the soya-bean meals, differed markedly in their behaviour in the small intestine. α-Protein and raw soya-bean meal induced a greater accumulation of soluble N than did heated soya-bean meal. Heated soya-bean meal gave only slightly higher levels of soluble N than casein, which gave low contents of both soluble and insoluble N. There were marked differences in the amount of insoluble N after raw and heated soya-bean meals had been eaten.

Composition of the intestinal soluble N fraction. In Table 4 are given the contents of 'dissolved proteins', 'peptides' and 'free amino acids', expressed as leucine equivalents, in the pooled intestinal contents of rats fed the various diets, and of fasted rats. In the control (fasted) rats the N was largely present as 'dissolved protein' with smaller amounts of 'peptides' and 'free amino acids'. For the purpose of comparison these quantities were taken as being representative of the endogenous secretion of N and

Table 4. *Amount of 'proteins', 'peptides' and 'free amino acids' (in mg leucine equivalent per 100 g body-weight) in the soluble nitrogen fraction of the pooled contents of the small intestine of six rats, 1, 2, 3 and 4 h after ingesting different proteins*

Diet	Time (h)	'Proteins'	'Peptides'	'Amino acids'
Control	—	3·76	3·83	5·75
Casein	1	3·67	5·91	9·42
	2	4·84	8·09	9·91
	3	3·27	4·08	6·00
	4	4·19	4·16	6·90
α-Protein	1	6·78	18·3	19·8
	2	6·44	22·2	22·2
	3	7·54	25·4	12·6
Raw soya-bean meal	1	12·1	6·67	16·6
	2	12·9	8·00	19·6
	3	13·6	6·77	16·5
	4	10·8	7·51	12·1
Heated soya-bean meal	1	7·02	9·70	13·5
	2	3·03	5·20	6·28
	3	3·49	6·47	6·86
	4	3·99	6·13	8·06

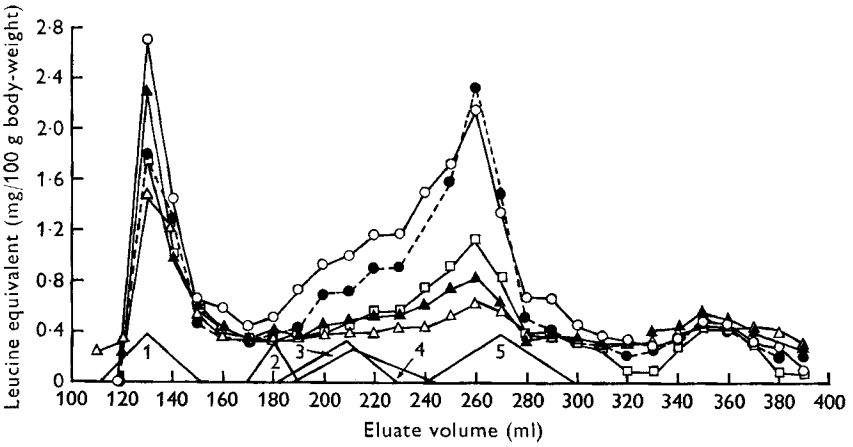

Fig. 1. Fractionation in Sephadex G-25 of the soluble nitrogen of the intestinal contents of rats 1, 2, 3 and 4 h after ingesting casein. α-Amino N was measured by reaction with ninhydrin after hydrolysis of the fractions with acid. Values are expressed as leucine equivalents/100 g body-weight. (●—●, 1 h; ○—○, 2 h; □—□ 3 h; ▲—▲, 4 h after feeding; △—△, fasted rats.) The numbered triangles on the abscissa depict the positions of the 'markers' in the effluent: 1, cytochrome c, mol. wt 12400; 2, insulin B chain, mol. wt 3483; 3, polymixin B sulphate, mol. wt 1447; 4, bacitracin A, mol. wt 1470; 5, a mixture of eighteen amino acids.

as a 'blank' or base-line correction in considering the findings for animals given the test proteins.

Fig. 1 shows the values for the intestinal contents of rats at 1, 2, 3 and 4 h after a test meal of casein. (For comparison the control curve is also reproduced to scale.)

As shown in Table 2, the total amounts of soluble N in the small intestine varied at the different times. In Fig. 1 and Table 4, therefore, the results are expressed as leucine equivalents per 100 g body-weight.

When casein was fed the contents of soluble 'undigested protein' and 'peptide'

reached a peak at 2 h after feeding and thereafter declined until at 4 h the values were little higher than in the fasted rats. The contents of 'free amino acids' was at a maximum at 1 and 2 h and thereafter declined rapidly to a low value at 4 h when the stomach had almost emptied. The proportions of 'protein', 'peptides' and 'free amino acids' per mg of N present changed with time—the amounts of 'protein' per mg N increased with time and reached a peak at 4 h after feeding, while the proportion of 'free amino acids' declined to a low point after 4 h. Peptide levels were highest at 2 h, and at 1, 3 and 4 h the levels were lower, and similar. The levels of 'protein', 'peptide' and 'free amino acids' per mg N at 4 h after eating casein were very similar to those in fasted rats.

As is evident in Table 4, there was a widely different distribution of N found in animals given α-protein instead of casein. The rate of disappearance of the undigested soluble protein component was markedly slower than with casein and the absolute amounts of N present in all the fractions were much higher, despite the finding that the rate of stomach emptying was more rapid with α-protein than with casein. Evidently the rate of digestion of α-protein was much slower. This slowness in digestion was also apparent in the content of peptide, which at 2 and 3 h after feeding reached a very high level.

More surprisingly, very high values were also found in the 'free amino acids' fraction. This raises the interesting question of how to account for the apparent slowness of absorption of the 'free amino acids' released during proteolysis. A possible explanation lies in the suggestion of Buraczewski *et al.* (1967) that the presence of a large accumulation of undigested peptides saturates the absorption sites involved in the transport of amino acids across the mucosal barrier and so blocks amino acid intake.

In the course of digestion of raw soya-bean meal, a very high content of undigested soluble protein was found which remained almost unchanged during the 4 h period (see Table 4). It is well established (Lyman & Lepkovsky, 1957; Haines & Lyman, 1961; Guggenheim & Goldberg, 1964; Barnes & Kwong, 1965; Barnes, Kwong & Fiala, 1965) that ingestion of raw soya-bean meal stimulates greatly increased secretion of enzyme protein into the small intestine and causes hypertrophy of the pancreas. At the same time the reabsorption of endogenous N is inhibited (Kwong, Barnes & Fiala, 1962). Thus the high values we obtained for the undigested 'soluble protein' fraction were probably largely comprised of endogenous protein. The 'peptide' fraction was small in amount. 'Free amino acids' were at a maximum at 1 and 2 h, and thereafter declined. The general picture obtained with raw soya-bean meal was strikingly different from that with α-protein.

When heated soya-bean meal was given, however, the soluble protein component was at a peak at 1 h after feeding and then progressively decreased until, at 4 h, the net content was zero. As with the untreated product, the peptide fraction was in low concentration. The 'free amino acid' fraction was at a peak at 1 h after feeding and thereafter declined much more rapidly than the corresponding fraction from raw soya-bean meal.

General discussion

There were wide differences between the different proteins in their rate of passage through the digestive tract and in their course of digestion. Raw and heated soya-bean meal did not differ in their rates of passage through the stomach, but differed greatly in their digestion in the small intestine. The larger accumulation of insoluble N in the small intestine of animals given raw soya-bean meal may be due to greater amounts of mucosal debris sloughed-off during the passage of food. Alternatively, it may reflect the slower rate at which raw soya-bean meal dissolves in the digestive fluid. It is of interest that de Muelenaere (1964*a*) found raw soya-bean meal to be more soluble than heated meal. In a later paper he showed that the amounts of insoluble protein present in the small intestine of rats given an amino acids diet supplemented with raw trypsin-inhibitor were large compared with the amounts present when heated trypsin-inhibitor was given (de Muelenaere, 1964*b*). The high proportion of insoluble N seemed to be largely contributed by sloughed-off mucosal debris.

The soluble N in the intestine of fasted rats contains mainly 'undigested dissolved protein'. A similarly large proportion of 'dissolved protein' was found when raw soya-bean meal was given. From the results of de Muelenaere (1964*a*) and the present findings it seems probable that the large accumulation of 'dissolved undigested protein' in intestinal contents of rats given raw soya-bean meal was due in the main to an enhanced secretion of endogenous protein stimulated by the raw soya-bean meal.

Casein and α-protein are broadly alike in their solubility, digestibility and biological value. Thus for these two proteins, the wide differences evident in their course of digestion are not paralleled by similar wide differences in the biological utilization of their N.

It is evident that proteins which digest at widely different rates may be equally efficiently utilized. Ultimately, of course, slow digestion must result in loss of N in the faeces, as was evident in the findings of Buraczewski *et al.* (1967) for heat-damaged fish proteins. There are important implications in this situation in relation to the concept (Melnick, Oser & Weiss, 1946) that for efficient utilization, amino acids liberated during digestion must appear simultaneously at the sites of synthesis within the tissues. Further discussion of this topic can best be deferred to a later paper in which the amino acid composition of all the different fractions will be presented.

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