Conclusion

This series of experiments is consistent with the hypothesis presented that NH_3 diffuses rapidly from the rumen and that a portion of this ammonia passes into peritoneal fluid and reaches the jugular vein without traversing the liver.

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Some aspects of the digestion of proteins

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Recent developments in analytical techniques have allowed more detailed studies to be made of the course of protein digestion. In this paper we consider some aspects of studies on the extent to which the behaviour of a food protein in the gastro-intestinal tract may be related to its digestibility and amino acid composition, and, ultimately, to its biological usefulness. Wider aspects of protein digestion have been reviewed by Gitler (1964), Rogers & Harper (1966) and Crane (1969).

Proteins differ in their susceptibility to digestion but the principal stages have been clearly established. Ingested food is stored in the stomach where it is moistened, softened and mixed with HCl and pepsins, the combination of which results in denaturation of native proteins and their partial or complete solubilization. The chyme passes into the duodenum as protein or large peptides where it is subjected to the sequential action of the peptidases deriving from the pancreatic and intestinal juices, yielding fragments which may be absorbed by the intestinal mucosa and made available to the animal through the portal circulation. There are considerable differences in the time-course of the breakdown and assimilation of individual proteins and even more marked differences after damage by heating.

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The role of the stomach

The stomach plays an important part in the regulation of digestion in acting as a temporary storage organ, reducing the meal to a pH value of about 2, and initiating the process of digestion. It also acts as an osmotic shield (Hunt & Pathak, 1960) to prevent the passage into the intestine of large volumes of hyperosmotic material.

The rate of emptying of the stomach after a meal is influenced by a variety of factors. It is affected by the nature and level of the protein and by the composition of the diet as a whole. The physical state of the meal is also important; liquid or finely divided meals leave the stomach more quickly than do coarser meals (Marcus & Lengemann, 1962). Wide variation in the rate of stomach emptying may be found even in the healthy animal under the influence of emotional states such as fear or excitement. The length of the prior period of starvation may also affect the transit time (Danhof, 1961), and Buraczewski, Porter and Rolls (unpublished results) found that the restriction of bodily movement by confinement in anticoprophagy cages delays stomach emptying, and training animals to eat for short daily periods results in an increase in the rate of passage of food.

In general, increasing the quantity of diet fed at a single meal increases the absolute rate of stomach emptying, but reduces the rate as a percentage of the diet fed. The rate of emptying depends on the type of protein fed (see Table 1) and appears to be unrelated to the nutritive quality of the protein (Rogers & Harper, 1966; Zebrowska, 1968).

Table 1. *Percentages of nitrogen and dry matter* (DM) *remaining in the stomach of rats 2 h after iso-nitrogenous meal of protein* (24·3 *mg N*/100 *g rat*) *alone and with carbohydrate* (167 *mg*/100 *g rat*)

Diet	N	DM
Whole egg protein	23	23
Zein	26	32
Gluten	25	28
Gelatin	2	3
Amino acid mixture	23	29
Casein	35	35
Casein + maize dextrin	39	30
Casein + glucose	41	32
Casein + soluble starch	42	41
Casein + sucrose	51	43
Casein + lactose	58	45

The passage of proteins from the stomach is affected by the presence of other dietary constituents, particularly carbohydrate and fat (Rosenthal & Nasset, 1958; Peraino, Rogers, Yoshida, Chen & Harper, 1959). Buraczewski, Porter, Rolls & Zebrowska (1970) found that when different proteins were fed with the same carbohydrate the nature of the protein component largely determined the rate of emptying, whereas the effect of feeding different carbohydrates with a particular protein could be related to the properties of the carbohydrate: very soluble carbohydrates, especially those which were poorly absorbed such as lactose, tended to delay stomach emptying (Table 1).

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Apart from its storage and regulatory functions, the stomach initiates protein digestion through denaturation by HCl and preliminary proteolysis by the gastric enzymes, of which the major constituent is pepsin, thus facilitating attack by intestinal proteases. By fractionation in Sephadex gel of the soluble stomach contents of rats given protein meals, Buraczewski, Rolls and Porter (unpublished results) found that they consisted almost entirely of soluble protein and large peptides. Amino acid levels were low. The proportion of peptide material was dictated not only by the digestibility of the protein but also by the rate of stomach emptying; thus, whole egg protein, although readily soluble, was present mainly as protein, whereas casein, which remained longer in the stomach, gave higher relative peptide levels and more small peptides. Gluten gave a preponderance of soluble protein whereas unsolubilized diet remained in the stomach, but as digestion proceeded the rate of stomach emptying slowed down, permitting further digestion, and the proportion of peptides increased.

These findings, together with the rapid initial rate of stomach emptying, the delay in reduction of gastric pH and the low pH needed for efficient proteolysis, indicate that a large meal of protein leaves the stomach preponderantly as protein and very large peptides. To the extent that peptic digestion is of importance in the utilization of proteins it would seem that efficiency of digestion would be favoured by frequent small meals rather than infrequent large ones.

Protein digestion in the intestine

In the small intestine, the chyme is mixed with the bile, its pH is raised and the proteins are attacked by a variety of proteases and peptidases secreted by the pancreas and the intestinal tract in response to the nature of the diet, particularly to its protein content (cf. Abdeljlil, Visani & Desnuelle, 1963; Twombly-Snook & Meyer, 1964). Although these enzymes are themselves digested and reabsorbed, they are protected by the presence of exogenous proteins which act as preferred substrates and are digested more rapidly.

Nature of the products of digestion. Investigations of the process of digestion by analysis of intestinal contents show that, due to the very rapid absorption of dietary nitrogen, the feeding of a well-digested, balanced protein results in little more accumulation of nitrogen in the distal small intestine than occurs after starvation or the feeding of a protein-free diet, despite the rapid passage of food from the stomach. However, with less well-digested proteins the nitrogen levels in the small intestine are dependent on the protein fed, and notably higher levels may be found after feeding raw soya-bean meals, poorly digested proteins or proteins which have suffered damage during extraction or by heat treatment (Table 2) (Buraczewski, Buraczewska & Ford, 1967; Zebrowska, 1968). Although solubility and digestibility do not necessarily coincide, poor solubility may be a factor when a protein such as zein is fed (Chen, Rogers & Harper, 1962).

By filtration in Sephadex gel it has been possible to separate the soluble nitrogenous material in the contents of the small intestine of rats into 'protein', 'peptide' and 'free amino acids'. When highly digestible proteins such as casein or cod-

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Table 2. *Nitrogen levels (mg/100 g) in the small intestine 2 h after feeding iso-nitrogenous protein meals (24·3 mg N/100 g rat)*

Diet	Insoluble N	Soluble N
(Fasted 18 h)	0·15	1·19
Maize starch	0·19	2·46
Casein	0·13	2·48
Cod meal – unheated	0·23	2·13
Cod meal – heated 135°, 18 h	2·07	5·73
Cod meal – heated 145°, 18 h	4·20	4·11
Raw soya-bean meal	0·97	5·29
Heated soya-bean meal	0·46	2·69
Zein	5·02	2·16

muscle protein were given, most of the α-amino-nitrogenous material was in the form of soluble protein and free amino acids with relatively smaller amounts of peptides. However, when less well-digested sources of protein such as heat-damaged cod muscle or heat-damaged fish meal were given, the amounts of all the fractions were greater and the proportion of peptides was markedly higher than after feeding the corresponding unheated material (Buraczewski *et al.* 1967; Zebrowska, 1968; Buraczewski, Porter and Rolls, unpublished results) (Table 3). Amino acid analysis

Table 3. *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 rats 2 h after ingesting different proteins*

Diet	'Protein'	'Peptide'	'Amino acid'
(Fasted 18 h)	2·0	1·4	3·1
Cod meal – unheated	2·1	2·3	6·8
Cod meal – heated 18 h at 135°	5·2	11·5	16·6
Cod meal – heated 18 h at 145°	4·6	8·4	10·6

of the separated protein and peptide fractions of the small intestine contents showed that the levels of the lysine and glutamic acid were often higher in the peptide fraction than in the original protein, a notable exception being casein, which is known to release its lysine readily (Rolls, 1970).

The presence in the gut of these enzyme-resistant (or even slowly hydrolysed) peptides may have nutritional significance beyond the immediately obvious loss in protein quality due to poor digestibility. It has long been known that amino acids must be simultaneously available at the sites of synthesis for their most efficient utilization (Melnick, Oser & Weiss, 1946) so that should the composition of these resistant peptides not reflect that of the original protein but concentrate certain amino acids, this could have a profound effect on the utilization of the protein. Lysine is particularly sensitive to reduction of availability by heat damage (Greaves, Morgan & Loveen, 1938; Hankes, Henderson, Brickson & Elvehjem, 1948; Carpenter, Morgan, Lea & Parr, 1962) and the unidentified peptide component is especially rich in this amino acid (Ford & Salter, 1966). Bjarnason & Carpenter (1970) have suggested that this diminished availability may be the result of reaction between the ε-amino group of lysine and the amide group of asparagine or glutamine.

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Since lysine is not only an essential amino acid but often one whose biological availability limits the utilization of the protein, this can be a serious factor.

The efficiency of the digestive process in the small intestine in keeping the nitrogen levels low after protein meals has led to suggestions that hydrolysis is not normally a rate-determining factor in the absorption and utilization of proteins. Experiments comparing the uptake of protein and amino acids in dogs (Janney, 1915), of yeast protein and yeast protein hydrolysates in man (Crane & Neuberger, 1960), and of beef and an amino acid mixture in rats (Gupta, Dakroury & Harper, 1958) indicated that the rate of absorption was the same for intact protein as for the free amino acids. In all these experiments, however, low levels of readily soluble proteins were given and other workers, using differing experimental conditions, have found that hydrolysates were absorbed more rapidly than were intact proteins (Zimmermann-Nielsen & Schønheyder, 1962; Scharrer & Zucker, 1968). Certainly, the rate of hydrolysis cannot be unimportant when such poorly digestible protein sources as zein or severely heat-damaged fish meals are given and it may be of particular importance when considering the value and effect of supplementing deficient proteins with their limiting amino acids to enhance their nutritive quality. If there were any marked discrepancy between the rates of absorption of the amino acids of the protein and the supplemental amino acid, the efficiency of utilization of the supplement might be impaired.

Rolls (1970) found that the addition of supplements of the appropriate limiting amino acids caused no changes in the absorption of the proteins in rats but that, in general, the free amino acids were absorbed more rapidly than the protein-bound amino acids; with all the test proteins, excepting gluten, there was no indication, at least from changes in blood plasma amino acid levels, of increased tissue synthesis as a result of supplementation. It appears, therefore, that rate of hydrolysis might, under these circumstances, be a major factor in protein absorption. In these experiments large meals were fed to an animal that is normally a continuous feeder, and if smaller and more frequent meals had been given the efficiency of supplementation might have been improved. The findings that the free amino acid supplement is absorbed relatively quickly may explain reports that supplementation does not necessarily make the nutritive value of a protein equal to that of the model (e.g. Banks, Allison & Wannemacher, 1964; Hogan, Gillespie, Koçtürk, O'Dell & Flynn, 1955). However, it does not necessarily follow that it would be advantageous for a supplemented protein to be digested more rapidly. If the amino acids were made available faster than the rate at which they could be utilized in the tissues, they might be subject to wasteful deamination (Gitler, 1964; Nielsen, 1966).

The nature of the products absorbed. There is still uncertainty about the form in which the digested protein is absorbed. The classical view is that proteins are degraded completely to amino acids and it is certainly true that there are peptidases in the intestine capable of converting peptides to free amino acids and that high levels of free amino acids have been detected in the digesta (cf. Wright & Wynn, 1951; Zebrowska, 1968). Fisher (1954, 1967) has criticized this view on the grounds that observed in vitro rates of hydrolysis appeared too slow to account for known rates

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of protein absorption, but Gitler (1964) has pointed out that these calculations omitted many factors present in in vivo digestion.

Analysis of the portal blood has given no evidence of the presence of peptides, even when these had been shown to be present in the intestinal contents (Levenson, Rosen & Upjohn, 1959; Dent & Schilling, 1949; Parshin & Rubel, 1951; Dawson & Holdsworth, 1962; Dawson & Porter, 1962). The finding that amino acids could be absorbed more readily by the intestine from peptides than from free amino acids, not only from di-, tri-, and tetra-glycine (Craft & Matthews, 1968; Craft, Geddes, Hyde, Wise & Matthews, 1968), which may not relate to protein digestion (Rhodes, Eichholz & Crane, 1967), but also from mixed peptides (Matthews, Crampton & Lis, 1968; Craft, Crampton, Lis & Matthews, 1969; Matthews, Lis, Cheng & Crampton, 1969), indicated that small peptides may be an important component of the nitrogen absorbed from the gut. Further evidence for the absorption of peptides has been given by Nixon & Mawer (1970) who found in man that they could not account for the absorption of all amino acids from a protein meal in the form of free amino acids. But perhaps the most direct evidence for the normal simultaneous mucosal uptake of peptides and amino acids comes from comparative studies of intestinal absorption in normal subjects and in a case of Hartnup disease, a rare condition in which peptides but not free amino acids are absorbed (Asatoor, Cheng, Edwards, Lant, Matthews, Milne, Navab & Richards, 1970). The failure of the absorption of free amino acids in this condition is not reflected in impaired nutrition and the clear implication is that an important proportion of the food protein may be absorbed as peptide.

It is thought that, in general, peptides do not cross the intestinal wall intact (Agar, Hird & Sidhu, 1953; Newey & Smyth, 1959). The question whether proteins are absorbed as peptides or as amino acids may be partly semantic, but the actual site of peptide hydrolysis is still under investigation. The peptidase activity may lie in the brush border of the epithelial cell (Ugolev, 1965; Miller & Crane, 1961; Ugolev & Kooshuck, 1966) where the major leucine aminopeptidase has been located (Holt & Miller, 1962); although other workers favour the interior of the mucosal cell, hydrolysis occurring after the peptides have entered the cell intact (Newey & Smyth, 1960, 1962). Parsons (1971) has proposed a scheme by which adjacent absorption sites themselves act as dimerases and Matthews *et al.* (1969) have discussed the kinetics of absorption by model systems.

These considerations are relevant to the findings of Buraczewski *et al.* (1967) who showed that there was an accumulation of undigested peptides and unabsorbed amino acids in the intestinal contents of rats given heat-damaged fish meals. They suggested that the large accumulation of 'unavailable' peptide saturated the absorption sites that are involved in the transport of amino acids into the mucosal cells and prevented the uptake of amino acids.

Bunyan & Price (1960) determined the nutritive quality for the rat of a range of whale-meat meals which were broadly similar in their total amino acid composition. It is apparent from their results that the biological values of the meals varied roughly in proportion to their digestibility, indicating that not only was the digestibility

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of the poorer meals lower but that correspondingly lower percentages of the nitrogen absorbed from the gut were retained. These findings could be explained in terms of different patterns of amino acids being absorbed from the different qualities of meal but Ford (1964) has suggested the further possibility that some proportion of the amino acids absorbed during the digestion of these damaged meals might still be non-metabolizable and locked up in unavailable peptides which were excreted in the urine.

Recently, J. E. Ford and C. Shorrock (private communication) have investigated the possibility that some of this undigested or indigestible peptide material is absorbed intact and they have found that rats given heat-damaged proteins consistently excrete higher levels of peptides in the urine than do animals given the corresponding unheated proteins. Furthermore, the total urinary output of free and combined lysine, and to a lesser extent of glutamic and aspartic acids, was appreciably higher in the animals given the damaged proteins.

Plasma amino acid levels

It has been known for some time that the amino acid content of the blood, particularly of portal blood, rises after a protein meal (Howell, 1906; Folin & Denis, 1912; Van Slyke & Meyer, 1912). More recently, a number of workers have shown that considerable increases in plasma amino acid levels occur after feeding, and that these are always larger in portal than in systemic blood (e.g. Peraino & Harper, 1963; Wheeler & Morgan, 1958; Denton, Gershoff & Elvehjem, 1953; Porter & Williams, 1963). The rise may occur within 0·5 h of eating and may last for 6–8 h in the rat, dog and man (Frame, 1958; Levenson *et al.* 1959; Crane & Neuberger, 1960; Dawson & Porter, 1962).

The magnitude of the rise in plasma levels has been shown to depend on the nature and quality of the protein fed. Heat-damaged or indigestible proteins caused lower rises in portal levels than did unheated or easily digested proteins (Denton & Elvehjem, 1953; Wu, 1954; Wheeler & Morgan, 1958; Guggenheim, Halevy & Friedmann, 1960; Buraczewski, 1966). Rapidly digested proteins such as gelatin and gluten caused higher increases than did more slowly digested proteins, and the feeding of poorly digested or heat-damaged proteins produced falls in systemic blood plasma amino acids. The rise in portal plasma amino acid levels may also be reduced or delayed by feeding with the protein other dietary constituents which may affect the rate of stomach emptying or, as with carbohydrate, be preferentially absorbed (Denton & Elvehjem, 1954; Peraino *et al.* 1959; Orten, 1963; Rolls & Porter, unpublished results).

The portal plasma amino acid concentrations represent the amino acids available to the animal unaffected by synthetic activity other than that which has taken place in the wall of the gut. Correlations have been found between the pattern of increases after feeding in the levels of essential, but not non-essential, amino acids in portal blood plasma and the composition of the protein. Despite this, attempts to use these patterns to predict limiting amino acids and nutritive value for growth or maintenance

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have yielded discouraging or only approximate results and direct correlation of plasma amino acid levels with protein quality has not yet been achieved.

Conclusion

The importance of detecting heat damage in processed protein concentrates used for animal feeding is well recognized, as witness the increasingly widespread use of the test for available lysine (Carpenter, 1960) and the more recent development of other techniques for monitoring protein quality (cf. Ford, 1962; Bunyan & Woodham, 1964; Boyne, Price, Rosen & Stott, 1967).

The lower solubility in gut fluids and the slower rate of digestion of proteins damaged by heat result in a markedly lower nitrogen digestibility, and are associated with an abnormal accumulation of nitrogenous compounds in the small intestine. Higher levels of soluble protein may be due in part either to increased secretion of endogenous nitrogen (Zebrowska, 1968) or to delay in the digestion of endogenous nitrogen because of its protection by undigested food protein (Buraczewski, 1966), and may increase the loss of endogenous faecal nitrogen. The higher levels of peptides may interfere with amino acid absorption.

The extent to which the findings with proteins severely heat-damaged in the laboratory are important in human and animal nutrition is not yet known, but they do underline the importance of factors other than amino acid composition in determining the nutritive quality of dietary protein. For example, the implication that the inclusion of a source of heat-damaged protein in a diet containing good quality protein may reduce the digestibility of the latter should be tested experimentally.

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