Yet a level of 4–6 ppm seems necessary to reduce the incidence of widespread osteo-porosis and collapsed vertebrae in women (Underwood, 1970).

These are but examples of the complexity of the problem we are going to discuss.

I would remind members that a survey of the nutrient requirements of farm livestock has been published by the Agricultural Research Council:

(1) Poultry (Agricultural Research Council, 1963),

(2) Ruminants (Agricultural Research Council, 1965),

(3) Pigs (Agricultural Research Council, 1967).

These are not presented in the form of practical allowances. Hence, in practice, safety margins will be allowed to take account of such factors as variations in availability of nutrients in different samples of feeding-stuffs, possible losses during manufacture and storage of food, breed and individuality of animals, environmental circumstances and standards of husbandry.

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Endogenous losses of nutrients

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Endogenous losses from the body occur via the urinary system, the skin, the lungs, the mammary gland (in lactating animals) and via the alimentary tract. It is com-paratively easy to determine the extent of the losses by the first four routes but many complications occur when the alimentary tract is considered. These are to do with the secretions that enter the tract and their subsequent reabsorption, the continued desquamation of epithelial cells that occurs, and the flora and fauna that inhabit the tract. The result of these complications is that it is difficult or impossible to dis-tinguish between endogenous and exogenous compounds or to form conclusions on whether the compounds reabsorbed confer less or greater nutritional benefits to the animal than the form in which they initially appeared in endogenous secretions.

It is essential to know the total losses that occur in the faeces when balance studies are undertaken, no matter whether they are assigned to 'endogenous' or 'exogenous' sources and any such assignment can only be arbitary, although these cannot give information on the exchanges that occur within the alimentary tract. A further complication is that the secretions into the alimentary tract and the density of the microflora are influenced by the nature and the quantity of the food eaten by the animal, and the latter has a pronounced effect on faecal losses of nitrogen.

The exchange of nitrogen in the alimentary tract

As the situation concerning protein is especially complicated in the alimentary tract I shall use this as an example (Fig. 1).
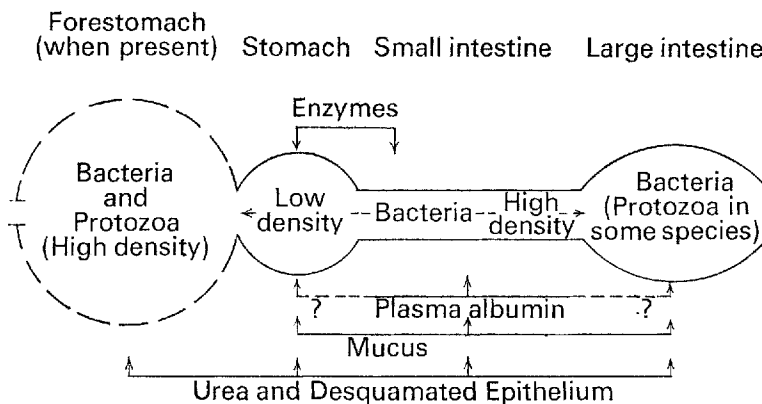

Fig. 1. Diagrammatic representation of endogenous nitrogen–containing materials entering the alimentary tract and the density of the microbial population.

The quantities of nitrogen secreted into the alimentary tract can only be roughly assessed and sufficient is known of them in sheep and in man to indicate that they are at least as great as the quantities consumed in the food. Thus, Wilson (1962) considers that the contribution made by desquamated epithelia alone in man may amount to about 25 g protein. In the sheep the fundic and pancreatic secretions may contribute about 2–5 g nitrogen and the small intestine may contribute a further 7–9 g most of which can be precipitated by trichloroacetic acid.

No part of the alimentary tract is sterile and this is vouched for by Williams-Smith (1965) who examined the alimentary content of a wide range of animals including the monkey, dog, cat, horse, pig, rabbit, guinea-pig, rat, mouse, hamster, fowl and duck, for *Escherichia coli, Clostridium welchii,* streptococci, lactobacilli, yeasts and bacteroides. Not all the animals examined contained all species of micro-organism. In general, the viable counts per g of contents were greater in the cardiac part of the stomach than the pyloric part; they increased in the upper small intestine and then decreased towards the ileum to attain their highest concentrations in the large intestine. This is a familiar picture in the ruminants with the exception that the concentrations are usually greatest in the rumen.

Nitrogenous compounds that enter the alimentary tract are exposed to enzymes of bacterial as well as endogenous origin. Owing to the speed of action of pepsin and trypsin it seems that hydrolytic and rapid digestion of ingested protein to amino acids predominates in the simple stomach and upper small intestine. Dietary protein is denatured by cooking or exposure to the acid environment of the stomach and appears to be more susceptible to peptic and tryptic activity than endogenous protein (Crane, 1964); in any event there is evidence from the studies of Nasset & Ju (1961), who gave [^{14}C]casein to dogs with jejunolostomies, that labelled nitrogen attributable to casein rapidly disappeared from the intestine and the proportion of endogenous to exogenous nitrogen exceeded the exogenous nitrogen ingested within 2·5 h by 75 to 90%. Many experiments with rats have been done in which large dilution of exogenous nitrogen by endogenous nitrogen has been found in the upper intestine; these experiments, however, are subject to distortion owing to the rapid shedding of epithelial cells that occurs on death (Badawy, 1964). Very large increases of endogenous nitrogen, a considerable part of which was attributable to mucus, have been found by Bolton (1964) in the duodenum of the fowl. Increases in nitrogen in the upper intestine of slaughtered sheep (Badawy, Campbell, Cuthbertson, Fell & Mackie, 1958) can still be detected in living sheep in which duodenal contents were taken through a permanent cannula located immediately caudal to the pylorus and compared to contents taken from a cannula situated at the other end of the duodenum. The increase, however, was less than occurred in slaughtered sheep. Anaesthetized sheep that are not exsanguinated also show less increase than those that are shot or anaesthetized and then bled (Badawy, 1964).

Secretion of mucus occurs throughout the length of the gut in simple-stomached animals. In ruminants salivary mucus is the only form that enters the rumen. Hecker (1967) has made a rough assessment of the relative incidence of mucus secreting glands throughout the gut of sheep by counting cells in histological preparations that show when staining by the periodic acid Schiff method the presence of mucus. His results show variable but considerable numbers of 'mucus'-containing cells in the abomasum, regular but small numbers in the small intestine, and increasing numbers in the large intestine which reach a peak at the second part of the colon. Estimates of mucus in the contents of the alimentary tract of sheep determined by the hexose and methylpentose sugars present in soluble mucus show that their concentrations in the contents of the ileum and large gut are considerably greater than in the upper small intestine and abomasum. Rhamnose and fucose, separated by paper chromatography, also increase in the same way. Only a few bacteria contain fucose in their cell wall constituents. Owing to the complexities of mucus and to the fact that its physical state rapidly alters, so that insoluble mucus becomes soluble, these methods can only give an approximate indication of the concentrations present. As there is little soluble mucus present in either the rumen or abomasum, and large concentrations in the ileum and caecum, the values are not merely reflecting bacterial cell wall constituents. In vitro experiments with isolated mucin showed that little or no breakdown occurred in rumen liquor, variable but sometimes considerable breakdown occurred in the ileal contents but considerable breakdown occurred in caecal

liquor and in faecal extracts during 24 h incubation.

The question whether enzymes as such are degraded as they pass through the alimentary tract is obscure. Proteolytic activity is normally present in faeces although whether it is due to trypsin appearing in the faeces or to proteolytic enzymes of bacteria is not clear. Maybe it is the result of both. There is strong evidence that tryptic and chymotryptic molecules are altered as they pass through the intestine of rats; it may be that they lose much of the amino-acid chain and the active part is retained. In contrast, there is evidence that proteolytic enzymes are rapidly inactivated in the small intestine (Pelot & Grossman, 1962). Owing to small quantities of nitrogen excreted in the faeces in relation to the suspected large quantities that enter in the form of protein it seems probable that most of the secreted enzymes are destroyed and that the products are reabsorbed.

Plasma albumin has been shown to enter the small intestine of sheep, rats and mice but there is no general agreement on how extensive this may be. In man about 12 g of plasma albumin disappear every 24 h; about 30% is broken down in the liver and kidney, but 9 g (70%) is unaccounted for. Some enters the stomach and intestine and appears in bile, but there is no agreement whether passage into the alimentary tract can account for the whole residue. As removal of a large part of the small and large intestine does not cause a marked diminution in the rate of catabolism of albumin, entry into the intestine does not appear to be the main route of loss (Freeman, 1964).

As so many different kinds of protein enter the alimentary tract it seems that they should be regarded as a loss to the body if their degradation is due to bacterial activity which will produce neither amino acids nor sugars. Instead, ammonia, short-chain fatty acids and traces of amines are likely to be the products of their disintegration unless their constituent elements are trapped within bacteria. As bacteria are ubiquitous in the alimentary tract, even though their concentrations differ in different parts, the chances of bacterial degradation are considerable or even inevitable when endogenous protein reaches the large intestine. In sheep we have found about 3 g amino-nitrogen to enter the large intestine from the ileum every 24 h and a loss of about 1 g nitrogen occurs between the ileo-caecal junction and the faeces. Unknown quantities of mucus, urea and shed epithelial cells enter the large intestine from the mucosa so that absorbed nitrogen must be greater there than 1 g. There is little doubt that the nitrogen of these compounds is absorbed mostly as ammonia.

Losses due to fermentative activity in the alimentary tract should be taken into account in any assessment of the endogenous losses of protein. It is tempting to assume that the difference between the minimal protein needs of the animal, represented by the minimum nitrogen output in the urine when animals are on an energy-adequate diet with little or no protein and the actual protein needs, represents the loss of amino acids due to bacterial degradation of endogenous protein secreted into the alimentary tract.

Thus, if we accept that 2 g nitrogen represents the minimal excretion of a 70 kg man for tissue catabolism, this represents 12·5 g protein. To maintain nitrogen

equilibrium a dietary intake of about 45 g protein per d is considered to be necessary. Is it reasonable to suppose the additional 32·5 g protein is needed to compensate for endogenous protein secreted in the alimentary tract which is subject to bacterial degradation so that the absorbed products is ammonia rather than amino acids? One may disagree with the figures if the principle is acceptable.

The situation, however, is unlikely to be as simple as this. Although ammonia is likely to be the principal product absorbed as a result of bacterial degradation, this may be metabolically useful in the synthesis of non-essential amino acids providing that the necessary carbohydrate is eaten. The concept would be better applied to essential amino acids, but as the content in a good dietary protein and endogenous protein will be roughly the same as in body tissues this does not alter the suggested concept.

The significance of urea recirculation

About 25% of circulating urea enters the alimentary tract in man and as a result of its rapid hydrolysis gives rise to ammonia and carbon dioxide. Ammonia may be reabsorbed or incorporated into bacterial cells. In the monogastric animal it is hard to see how this recirculation has any particular benefit to the animal beyond supplying an additional source of nitrogen for bacterial growth. This may result simply in greater faecal loss of nitrogen. The situation with the ruminant is different for then urea entry into the rumen encourages bacterial growth and so assists in digestion. When the diet is inadequate in nitrogen this confers a benefit to the animal as it increases appetite and, therefore, the intake of energy.

The role of bacteria in causing loss of nitrogen via the alimentary tract should not be overlooked. Bulky foodstuffs with much fibre lead to greater losses of nitrogen in the faeces; this is usually ascribed to the abrasive effect of such diets on the mucosa but it could also be due to larger quantities of fermentable substrate reaching the large intestine. Similarly, excessive starch or inulin reaching the large intestine gives rise to extra bacterial growth and loss of faecal nitrogen.

Similar trends occur in the ruminant and, even though many of the recognizable organisms disappear in the small intestine, diets that increase bacterial growth in the rumen also increase the output of faecal nitrogen (Blaxter, 1964). It is not possible to ascribe all this increase to greater growth in the large intestine although this may be partly true.

The complications of the total situation are such that no simple method of allowing for endogenous loss within the alimentary tract can be put forward beyond the suggestion already made.

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Problems in the assessment of vitamin deficiency

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The modern British doctor may never wittingly have seen a patient with vitamin deficiency due to primary malnutrition and, unless he is the product of a medical school that encourages its students to spend an elective period in one of the developing countries, he may feel that talks on vitamin deficiency states are too unreal and academic to deserve his attendance. This is a measure of the state of our economy and of the successful policies of our nutritional experts. Those who have seen the pitiful figures in the cities or villages of large areas elsewhere in the world must view such matters in an entirely different light. However, the present account is concerned more with the problem in the United Kingdom, and particularly with the various factors concerned with the intake and availability of vitamins. From the practical point of view our clinicians may be faced with disorders arising from depletion of ascorbic acid, folic acid or calciferols. The existence in this country of patients with primary deficiency of other vitamins is more controversial. In the first instance, however, the subject will be considered in general terms.

Vitamin intake and requirements

It is not necessary to discuss here the precise amount of each of the vitamins that is believed to be required by the human male and female at different ages but, instead, the various factors that may affect the amount actually available to the body merits further consideration. At the same time, passing mention may be made of the major, general causes of individual variation in requirements. These matters in themselves could be the subject of a very long lecture or article, but may be compressed into a table, with a few added comments. Recently, I made use of an alphabetical list of aetiological factors of importance when discussing deficiency states in the elderly (Girdwood, 1970). At the risk of appearing to be lacking in imagination in my approach to the subject, I have set out Table 1 in a similar manner.