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THE BIOLOGICAL VALUE OF PROTEINS IN MIXED GRASS HAYS

(met een samenvatting in het Nederlands)

by/*door*

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INTRODUCTION

Proteins are essentially required for the building up of animal tissues and for the regulation of the vital processes; if they are lacking in the diet life appears to be impossible. Plants can build up proteins from simple nitrogenous constituents of the soil and the air with the aid of solar energy, while animals cannot perform such a process and depend chiefly upon plants for their protein supply. Only ruminants have been shown to utilize under certain conditions urea and other simpler nitrogenous compounds for protein formation which is mainly attributed to the activity of microorganisms in the reticulo-rumen.

The determination of food protein required for a specific function such as maintenance or growth or any other physiological function must not only be based on the quantity of protein but also on its quality since, from experimental data it has been concluded, already more than a century ago, that certain proteins fail to support growth and even may be insufficient for maintenance. Later on it has been shown that this is not so because they are provided in small quantities, but because they are qualitatively inadequate in amino acid composition. A prerequisite for the support of maintenance, growth etc., is namely that all the essential amino acids should be present in adequate amounts.

Yet, the nutritive value of a given protein should not be measured by its amino acid composition alone. For it is evident that merely the amino acids in the digested part of the protein are decisive, and not only the quantities of each of them but also the mutual ratios.

This makes it difficult to predict the nutritive value of proteins on the basis of amino acid composition. For the present biological experiments with animals to determine the "biological value" of food proteins cannot yet be dispensed with, even not in the nutrition of men and monogastric animals.

Still much more difficult is the problem of the nutritive value of proteins in ruminant nutrition, because of the processes of degradation and synthesis of proteins by the rumen bacteria.

The present paper comprises an investigation with sheep on the biological value of proteins in grass hay. Experiments of this kind have not yet been done in the Netherlands, although hay is a predominent winter feed. The first part contains a discussion and criticism on what has been found in general about this subject in the literature (chapter I to VI). The second part (chapters VII and VIII) describes the experiments and discusses the results secured. The summary and conclusions are presented in the third part.

PART I. DISCUSSION OF THE LITERATURE

CHAPTER I

ESSENTIAL AND NONESSENTIAL OR INDISPENSABLE AND DISPENSABLE AMINO ACIDS

The word protein has been introduced in the literature in the year 1838 by the scientist G. J. MULDER from the Netherlands. This word was suggested to him by the Swedish investigator BERZELIUS. MULDER was of the opinion that all kinds of albuminous substances mainly consist of the same nucleus or root-substance, the "proteine". Of course MULDER did not overlook the fact that the

various substances differ in their chemical and physical properties. He thought that this could be explained by the union of protein with different quantities of S and P (and O).

MULDER already considered protein to be a complex substance consisting of some heterogenous organic compounds, leucine being one of them. This substance had already been discovered in 1819 by PROUST and had been named leucine by BRACONNOT a year later (1820).

It is well known that later on many other amino acids have been discovered in proteins; however, it took more than a century before all the more important amino acids had been isolated, threonine being the last one (ROSE, 1938). This discovery enabled ROSE to solve a problem that had defied the efforts of even the most skilled investigators for 25 years.

As far back as 1912 ABDERHALDEN had tried to substitute amino acid mixtures for proteins. He incorporated 16 different amino acids in a diet using dogs as experimental animals. The animals soon contracted diarrhea, they vomited and refused to eat much of the food. The real significance of this result was not clear. It was possible that it meant that proteins in the food are indeed indispensable. On the other hand it also turned out that amino acids are less palatable than intact proteins, and thus poor growth could also result from a lower feed intake rather than from any specific deficiency.

ROSE and his collaborators in 1930 started a brilliant series of studies, in which they succeeded in substituting protein by an amino acid mixture in the diet of growing rats, so that the above mentioned problem was solved (1938). It was made clear that animals can live and grow with amino acids as the only nitrogen source.

In continuing their experiments they obtained definite results concerning a problem which was perhaps still older, namely the question which amino acids in the diet are indispensable and which are not. Although it had already been established that some amino acids, such as glycine and alanine, can be synthesized in the mammalian body, others, such as tryptophan, can not. For many other amino acids, however, the situation was not clear. With his feeding experiments on growing rats ROSE gave specific information as to the acids which cannot be lacked in the diet. He classified the amino acids occurring in food proteins as indispensable (essential) and dispensable (nonessential) amino acids. ROSE (1938) defined an indispensable amino acid as one which cannot be synthesized in the body out of the materials ordinarily available, at a rate sufficiently rapid to promote normal growth. A dispensable amino acid is one which can be synthesized in sufficient quantity out of the materials ordinarily available to the organism. In this respect he made the following classification of amino acids providing for maintenance and tissue building, as far as growing rats are concerned.

Indispensable amino acids: Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Dispensable amino acids: Alanine, aspartic acid, citrulline, cystine, glutamic acid, glycine, hydroxyglutamic acid, hydroxyproline, norleucine, proline, serine and tyrosine.

Arginine can be synthesized in the rat, but not rapid enough to meet the demands of normal growth. Hence, according to the above mentioned definition, it is an indispensable amino acid.

Lack of any one of the essential amino acids in the diet produces a negative

N-balance, stops repletion and causes the animal to lose appetite and to fall ill.

The results of ROSE have essentially been confirmed by his later work and by that of others. In his paper of 1948, however, he gives some additional information by mentioning that $^1/_6$ of the methionine requirement can be replaced by cystine and that $^1/_2$ of the phenylalanine requirement can be replaced by tyrosine; but in the absence of methionine or phenylalanine neither cystine nor tyrosine has a growth effect. Arginine can be replaced in part for growth by proline or glutamic acid but not by hydroxyproline.

The above mentioned paper also shows that the essential amino acids are not able to replace the total mixture of indispensable and dispensable amino acids completely. The growth of rats was namely definitely somewhat better when a mixture of all the amino acids was given. It has not even been shown conclusively that a mixture of indispensable and dispensable amino acids is able to substitute the natural proteins in all respects, because it turned out that the caloric intake on diets with only amino acids as nitrogen sources should be higher than normal.

ROSE's next step was to consider the quantitative requirements of amino acids for the growing rat. Later on, other investigators made similar experiments with pigs, chickens, infants and even with honey-bees. Moreover, experiments on requirements for maintenance were performed. The results of these investigations have been collected in table 1.

In order to make the results more comparable we calculated the percentage distribution, assuming the sum of the most important essential amino acids to be equal to 100%, thus without arginine and glycine.

Although there are some differences, the agreement between the columns is striking. Therefore one must conclude that the different species require essentially the same pattern of amino acids.

These differences however are worthy of closer examination. From table 1 it can be seen that all species require all the essential amino acids. Only histidine does not seem to be necessary for adult humans. This conclusion has however been challenged. NASSET *et al.* (1954) pointed out that the apparent nonessentiality of histidine to the adult human male as reported by ROSE *et al.* (1951) may be associated with the relative short feeding periods used by these investigators and with the possibility that humans utilize endogenous histidine derived from degradation of haemoglobin.

In the growing animals histidine and arginine are essential. The growing chicken occupies a unique position in that it requires glycine in addition to the other ten essential acids. The need for glycine and arginine in relatively large amounts may be due to feathering (HEGSTED *et al.*, 1941). This seems reasonable since feather proteins contain high percentages of these two amino acids. Moreover, it is known that microbial activities in the intestine of the chicken are rather small so that synthesis of amino acids can only be very limited.

Furthermore it is seen from table 2 that MITCHELL's figure for the rat's phenylalanine requirement for maintenance (column 3) is considerably higher than the figures mentioned in the columns 1 and 2. The reason for this probably is that MITCHELL included tyrosine in his figure for phenylalanine.

Other differences between MITCHELL's figures and those in columns 1 and 2 may be due to the fact that MITCHELL did not determine the requirements of the amino acids separately. He determined the minimum quantities of several proteins and protein foods required for the nitrogen equilibrium in adult rats

TABLE 1. Requirements of indispensable amino acids for the different species of animals

Amino acids	Maintenance					Maintenance plus Growth				
	Adult rats			Women	Men	Young rats	Young chicken	Young pigs	Honey-bees	Infants
	(FRAZIER *et al.*) (1949) tryptophan equals 1	(NASSET) (1957) mg./day per kg.$^{3/4}$	(MITCHELL) (1959) mg./day	(LEVERTON) (1959) gm./day	(ROSE) (1957) gm./day	(ALMQUIST) (1959) (%) of diet	(ALMQUIST) (1959) (%) of diet	(ALMQUIST) (1959) (%) of diet	(DE GROOT) (1953) tryptophan equals 1	(ALBANESE) (1950) tryptophan equals 1
Arginine	–	–	21.9	–	–	0.2	1.20	0.30	3.0	–
Glycine	–	–	–	–	–	–	1.00	–	–	–
Histidine	1.0	6.7	7.8	–	–	0.4	0.30	0.30	1.5	–
Isoleucine	6.2	30.0	20.8	0.45	0.70	0.5	0.60	0.60	4.0	3.0
Leucine	3.6	16.0	34.3	0.62	1.10	0.8	1.40	1.20	4.5	14.0
Lysine	2.0	3.6	11.2	0.50	0.80	1.0	1.00	1.10	3.0	5.6
Methionine	3.3	40.0	19.1	0.55	1.10	0.4	0.45	0.37	1.5	2.8
Phenylalanine	2.7	18.0	40.3	0.22	1.10	0.7	0.80	0.70	2.5	5.6
Threonine	2.4	20.0	12.2	0.31	0.50	0.5	0.60	0.60	3.0	2.9
Tryptophan	1.0	4.4	5.6	0.16	0.25	0.2	0.20	0.20	1.0	1.0
Valine	4.5	18.0	23.6	0.65	0.80	0.7	0.80	0.60	4.0	5.4

TABLE 2. The relative percentage requirements of indispensable amino acids for the different species of animals (expressed as percentages of the sum of the essential amino acids without arginine and glycine)

Amino acids	Maintenance					Maintenance plus Growth				
	Adult rats			women	men	Young rats	Young chicken	Young pigs	Honey-bees	Infants
	FRAZIER *et al.*	NASSET	MITCHELL	LEVERTON	ROSE	ALMQUIST	ALMQUIST	ALMQUIST	DE GROOT	ALBANESE
Histidine	4	4	4	–	–	8	5	5	6	–
Isoleucine	23	19	12	13	11	10	10	11	16	7
Leucine	14	10	20	18	17	15	23	21	18	35
Lysine	8	2	6	15	13	19	16	19	12	14
Methionine	12	26	11	16	17	8	7	7	6	7
Phenylalanine	10	11	23	6	17	14	13	12	10	14
Threonine	9	13	7	9	8	10	10	11	12	7
Tryptophan	4	3	3	5	4	4	3	4	4	2
Valine	17	11	13	19	13	14	13	11	16	13

and men and took the smallest amounts of the amino acids in these quantities as the required amounts. It is however not impossible that some amino acids were present in all the proteins tested in amounts exceeding minimum requirements.

Further, from table 1, it may be seen that the maintenance requirements as suggested by LEVERTON (1959) for women are somewhat lower than those given by ROSE for men, both on the basis of total daily amount per kilogram. LEVERTON, however, pointed out that, here too, there were definite differences between the studies as concerns the techniques used. In the experiments with women the quantities have been determined necessary to keep the experimental persons on an average in nitrogen equilibrium, the latter defined as zone in which the nitrogen excretion is within 95–105% of the intake. For men, however, the requirements have been based on the highest amounts needed by any subject for a slight but distinctly positive nitrogen balance. This readily explains the lower values for women.

When we consider the percentage distribution (table 2) nearly all differences between men and women disappear. The proportions between the requirements are about the same except for phenylalanine, the requirement of which for women seems to be about $^1/_3$ of that for men. This can, however, be explained by the fact that tyrosine is not included in LEVERTON's figure for women, whilst in ROSE's figure it is. If we take LEVERTON's figures for phenylalanine and tyrosine together the discrepancy disappears completely.

Regarding the maintenance requirement of men as compared with the adult rat, MITCHELL (1959) concluded that the cystine-methionine requirement is relatively more intense for the adult rat than for either the growing rat or for the adult human. On the other hand, the lysine requirement seems to be much less prominent among the amino acid requirements of the adult rat than among those of the adult man. MITCHELL adds that the most probable explanation rests on the high cystine and low lysine content of hair and keratins (BLOCK *et al.*, 1947) and on the probability that the amino acid requirements of the adult rat are dominated by the requirement for hair growth. Indeed the figures for lysine in table 2 for humans are higher than those for rats. With methionine, however, the difference is not conspicuous. The reason for this is probably that cystine is included in the methionine figure for humans.

In comparing finally (table 2) the amino acid patterns required for maintenance and for growth plus maintenance of men and animals, it will be seen that they are very similar. It should however be pointed out that the requirement of lysine for maintenance is smaller than for growth plus maintenance, especially with the rat; the requirement of methionine is definitely higher.

All these smaller and greater differences, however, do not detract from the conclusion that the various species of animals studied essentially require the same pattern of amino acids. From this it reasonably may be concluded that the ruminant tissues also need about the same amino acid pattern. In chapter II it will be shown that it is not necessary that all these amino acids should be present in the same proportions in their food, but that rumen microorganisms change the picture in breaking down part of the amino acids of the feed and in synthesizing new amino acids for building up their body proteins. Decisive is therefore not the pattern of the amino acids in the feed ingested but that in the liquor passing from the omasum to the abomasum.

CHAPTER II

DEGRADATION AND SYNTHESIS OF PROTEINS AND AMINO ACIDS BY RUMEN MICROORGANISMS

INTRODUCTION

It is generally known that in monogastric animals the feed proteins in the stomach and in the small intestine are broken down to amino acids which are absorbed as such. Indeed in the caecum and colon the non-digested residues are subject to a fermentation by bacteria resulting in further breakdown of proteins and other substances. These processes in the caecum and colon have not been studied sufficiently but certainly they are of minor importance, at least in men and in animals such as rats and pigs. This makes it possible to predict roughly, from the amino acid pattern, the biological value (*BV*) of many proteins utilized in the nutrition of men, pigs and rats.

Although it is probable that the amino acid requirements of the living tissues of ruminants are about the same as those of monogastric animals, yet the *BV* of the feed proteins may be very different for these two classes of animals. It is further assumed and even probable that in ruminants the function of the abomasum and the more caudal parts of the intestine is not much different from their function in such well-studied monogastric animals as the rat and the pig, both as to what these animals require to absorb from their alimentary tracts and as to how they digest material entering the abomasum and passing further along the intestines (CHALMERS *et al.*, 1954, b). It is therefore highly probable that an arbitrary mixture of amino acids leaving the fore-stomachs and passing into the abomasum has for the ruminant about the same nutritive value as for a monogastric animal. Differences in *BV* of proteins between ruminants and monogastric animals are therefore mainly due to the fermentations, *i.e.* the microbiological activities in the rumen.

As far back as the eighties of the last century TAPPEINER (1883) recognized the significance of the *rumen bacteria* for cellulose fermentation and for the production of rumen gases. BRAHM (1926) did some experiments on artificial digestion by incubating an appropriate culture medium with bacteria from the rumen and from other parts of the intestinal tract, thus, with the aid of a primitive kind of artificial rumen. ANKERSMIT (1905) was the first to examine the species of the bacteria in the normal bovine intestine. He considered *streptococus acidi lactici* as the prepondering germ in the rumen. Furthermore he found OMELIANSKI's cellulose dissolving bacteria.

TAPPEINER (1891) also found that the non-protein-nitrogen-compound (NPN-compound) asparagine can be utilized by bacteria in the intestinal tract for building up their body proteins. It is well known that this finding gave rise to an immense number of publications in which was shown that this bacterial activity is of great importance for the protein metabolism of the animal and for the *BV* of the nitrogenous compounds in ruminant nutrition. This question will be discussed in detail later on.

In Berlin, after the First World War, MANGOLD's collaborators (among others, FERBER) (MANGOLD, 1929) made extensive investigations on *infusoria* in the rumen which had been discovered as far back as 1843 by GRUBY *et al.* In

sheep and goats on normal feed the number of rumen protozoa was rather constant, namely about 1 000 000 per ml. In sheep, on low protein rations rich in carbohydrate, the number decreased considerably. From this it was concluded that the infusoria have a definite requirement of protein. On the other hand, increasing the feed protein by soybean meal and blood meal up to levels higher than normal gave no increase in the numbers.

Already at that time (MANGOLD, 1929) it was an important question whether infusoria are able to cover their nitrogen requirement with non-protein-nitrogen (NPN). When experimental wethers were given a ration consisting of hay and starch the number of infusoria decreased. Addition of ammonium acetate gave no increase but a further decrease. After feeding again a normal ration the number rose to ordinary levels. Similar results were obtained in experiments with urea instead of ammonium acetate. It was concluded that NPN, at any rate urea and ammonium acetate, cannot be utilized by rumen infusoria.

Nevertheless, MANGOLD thought that the infusoria are of considerable nutritional importance because they would transfer the less digestible vegetable protein into easily digestible animal protein of infusoria. In this connection he spoke of "biologisch veredeln" of the protein, which means "to ennoble biologically". In our opinion, however, this interpretation of his findings is doubtful, as it has by no means been shown that the proteins are dissolved easier or in greater amount by the infusoria than by the intestinal enzymes. Moreover, if so, the transfer of plant protein via infusoria to the animals means an intermediate step in which there must be inevitable protein losses through the metabolism of the infusoria.

Recent development in the knowledge of the rumen function has largely taken place in Britain. This development was greatly stimulated by a group working during the Second World War at Cambridge under the leadership of the famous Sir JOSEPH BARCROFT. By the British and other investigations our knowledge on rumen function has greatly increased and has grown out to a special branch of physiology, usually called *rumen physiology*.

These recent investigations have confirmed many fundamental data on the rumen function and on the microbiological activities in the fore-stomachs which had already been discovered many years ago by German investigators. Several other new facts have been added. In our opinion one of the principal features of modern rumen physiology is the recognition that the activities of the rumen-organisms are quantitatively of much greater importance than formerly was believed.

Several excellent reviews have appeared summarizing the results and reviewing either special aspects or the whole field. We may mention especially the reviews of CUTHBERTSON *et al.* (1953) and of CHALMERS *et al.* (1954, b), the thesis of OYAERT (1955), the monograph of ANNISON *et al.* (1959) and in addition papers of several authors presented at the 123rd meeting of the British Nutrition Society (Proceedings **18** (1959) 97), all of which have been of considerable benefit in preparing this chapter.[1]

[1] For the newest developments see the paper of M. J. CHALMERS and that of D. LEWIS in the Nottingham "Symposium and Colloquium on digestive physiology and nutrition of the ruminant" (in the press).

"DIGESTION" OF DRY MATTER AND ORGANIC MATTER IN THE FORE-STOMACHS

Modern rumen physiology has shown that a large part of the dry matter of the feeds is "digested" in the fore-stomachs. This means that it is degraded and that the degradation products either are absorbed by the walls of the proventriculi or broken down to methane and CO_2 and lost, mainly by belching. There are many degradation products and intermediates, but only those originating from the proteins will be described in detail later on. Concerning the other constituents of the feed we want to confine ourselves to a discussion of the dry and organic matter as a whole.

Suggestions on the quantity of dry matter disappearing from the reticulo-rumen have been made by PALHEIMO *et al.* (1955), GRAY *et al.* (1958) and ROGERSON (1958) by slaughtering the animals. HALE *et al.* (1947) calculated the extent of the rumen digestion of lucerne hay in fistulated animals with the aid of the lignin ratio. The digestion coefficient for dry matter was 48%, corresponding with 85% of the dry matter digested in the whole intestinal tract.

OYAERT (1955) in sheep determined the digestibility of clover hay and of clover hay plus concentrate in all three proventriculi together. With the aid of a special kind of fistula (BOUCKAERT *et al.*, 1954) OYAERT succeeded in determining the quantity and composition of the digesta passing from the omasum to the abomasum. By making at the same time use of a marker (carbowax 4000) OYAERT (1955) calculated that 52 to 75% (average 64%) of the total dry matter had been digested in the three fore-stomachs. BALCH (1957), also making use of a marker, tentatively found in cows that on average for the diets studied, 64% of the total digested dry matter was apparently digested in the reticulo-rumen. The rations consisted of hay and of hay plus various amounts of supplements.

HOGAN (1957) and HOGAN *et al.* (1960) utilized methods which made it possible to leave out markers. They adopted the method of exteriorising in sheep the flow of food in the beginning of the duodenum. During the experiments the digesta were permitted to flow out of a canula inserted in the proximal end of the duodenum, and were collected in portions of about 50 ml. After sampling the remainder was poured back as soon as possible through a second canula inserted in the distal part of the duodenum that had been completely separated from the proximal end. By feeding 300 gms. of meadow hay and 200 gms. of concentrates per day, they found that 70% of the digestible dry matter ingested disappeared in the four stomachs. As it is generally agreed that little absorption takes place in the omasum, this absorption mainly must have taken place in the three proventriculi.

KAMEOKA *et al.* (1959) avoided the implications originating from abomasal digestion and absorption. They succeeded in making a quantitative estimation of the digestion in the proventriculi in goats by collecting all alimentary tract contents upon entry into the abomasum via a fistula establishment between omasum and abomasum. The omasal ingesta leaving the omasum canula were collected and weighed hourly, warmed at 40°C, and poured into the abomasum, 24 hours a day. In this way they found that 61.7 to 85.4% of the digestible organic matter of the rations consisting of hay or of hay and concentrates disappeared in the fore-stomachs. On two rations of a low quality hay of predominantly orchard grass and red clover with 11.9% crude protein in the dry matter the figures were 85.4 and 75.2% respectively.

All the above mentioned investigations show that about $^2/_3$ or $^3/_4$ or even

more of the digestible dry matter and organic matter in rations of hay or hay plus some concentrate is already broken down and disappears by absorption (and eructation) from the fore-stomachs.

DEGRADATION OF PROTEIN

The degradation of protein in the fore-stomachs is also much more extensive than formerly was believed. The question is complicated by the fact that besides degradation there may be a considerable degree of protein synthesis by bacteria.

The degradation process of the proteins in the fore-stomachs has been studied several times and it has been shown that ammonia is one of the principal end products (PEARSON *et al.*, 1943; MCDONALD, 1948, a). The theory of the intermediate steps is for a large part derived from studies with amino acid solutions incubated with washed suspensions of rumen bacteria. Therefore, no definite conclusions about the real course of degradation in the reticulo-rumen have been secured.

Nevertheless, it has been demonstrated (SYM, 1938; PEARSON *et al.*, 1943) that the rumen contents show proteolytic activity. Moreover, CHALMERS *et al.* (1954, b) and ANNISON (1956) demonstrated very small amounts of α-amino nitrogen. The latter also found that diffusible peptides increased after feeding. By these and numerous other findings proteolytic degradation into amino acids is clearly obvious.

The degradation of these amino acids has been studied by EL-SHAZLY (1952). By incubating casein hydrolysates under anaerobic conditions with washed suspensions of rumen bacteria he found as reaction products mainly NH_3, CO_2 and volatile fatty acids in roughly equimolecular proportions. Some of the volatile fatty acids had branched chains, probably originating from valine, leucine and isoleucine. There was no significant formation of H_2 or CH_4.

Although the end products were identified, the way in which the amino acids are broken down is not clear. Besides de-amination of single molecules, there is also de-amination by the so-called Stickland reaction, a mutual oxidation-reduction reaction of two amino acids in which reaction besides NH_3 two molecules of fatty acid are produced. An example of this reaction is given by ANNISON *et al.* (1959) in their interesting discussion of the amino acid degradation in the rumen. We also mention the papers of SIROTNAK *et al.* (1953, 1954) and of VAN DEN HENDE *et al.* (1959).

It is an important question whether the essential amino acids in the rumen give rise to the corresponding hydroxy- and keto-acids. In case these would be absorbed, the de-amination would not always mean a definite loss, as in the tissues resynthesis might occur. EL-SHAZLY (1952) examined this problem by making ether extracts of rumen liquor, after which he separated the volatile fatty acids and the non-volatile acids by steam distillation. He found that the latter never exceeded 2.5% (equivalent basis) of the total volatile fatty acids present. It is however doubtful whether by these results the question has been settled definitely.

As for the fate of nitrogenous end products it has been shown that the ammonia is absorbed from the rumen (MCDONALD, 1952). There is indeed a close correlation between NH_3 in rumen contents and in portal blood (LEWIS *et al.*, 1957). Absorption of amino acids from the rumen however could not be demonstrated (ANNISON, 1956).

It is generally agreed that the absorbed NH_3 is almost entirely converted to urea in the liver. The urea content of the blood is indeed increased by high NH_3-production in the rumen and might even give supplementary information about the efficiency of protein utilization (LEWIS, 1957).

As already mentioned besides breakdown there is also synthesis of proteins by the microorganisms in the proventriculi. For this synthesis they may utilize either amino acids or degradation products such as NH_3 plus energy-yielding organic materials. Furthermore, substantial quantities of urea contained in the saliva enter the rumen. Moreover, there is perhaps also diffusion of urea from the blood through the walls of the proventriculi. It is well known that in the rumen urea is easily converted to NH_3 and, therefore, is also available for bacterial protein synthesis. In spite of this, in most instances, there is a net loss of nitrogen in the fore-stomachs.

It is evident that this net loss has been studied thoroughly. This work has been summarized by CHALMERS *et al.* (1954, b), by OYAERT (1955) and by ANNISON *et al.* (1959). It appeared that easily soluble proteins such as casein and groundnut cake produce large quantities of NH_3, whilst the NH_3-production from the little soluble zein and from maize gluten is slight. Herring meal behaved in an intermediate way. Furthermore, casein hardened by drying produced much smaller quantities of NH_3 than without hardening. MCDONALD (1952), OYAERT (1955), HEAD *et al.* (1957) and ANNISON *et al.* (1959) showed that by adding starchy feeds to the ration the level of NH_3 in the rumen is reduced.

Some attempts have been made in order to obtain estimates of the *magnitude of the net N-losses* in the proventriculi. The results were very variable as might be expected.

GRAY *et al.* (1953, 1956, 1958) using lignin as a marker examined the intestinal contents of slaughtered sheep. The quantity of N reaching the abomasum and duodenum was calculated to be equivalent to nearly 100% of the dietary N for sheep fed on 800 gms. wheaten hay (with 1.1% N), only 65% on 400 gms. wheaten hay and 400 gms. lucerne hay (N in the diet 1.8%), as little as 48% on 800 gms. lucerne hay alone (2.9% N) and 117% on 500 gms. wheaten hay and 250 gms. wheat straw (N in the diet 0.7%).

In the experiments of HALE *et al.* (1940, 1947) with two rumen fistulated cows, the entire contents of the rumen were removed, weighed, mixed, sampled and returned immediately to the rumen after a representative sample had been taken. By feeding the cows on lucerne hay and using lignin as a marker they found that at 12–14 hours after feeding only 40.4% of the feed protein was recovered in the rumen.

From ROGERSON's figures (1958), obtained with slaughtered sheep and with lignin as a reference substance, it can be calculated that 64% of the crude protein in a low quality hay ration (6.9% crude protein) had reached the omasum; 36% of the crude protein and 84% of the digestible crude protein had been "digested" in the proventriculi.

BALCH's observations (1957) with rumen fistulated cows refer only to two of the proventriculi (rumen and reticulum), but are nevertheless interesting. With diets consisting entirely of grass hay the amount of crude protein leaving the reticulo-rumen often appeared to be at least as great as the amounts ingested as food, suggesting that there was little or no absorption of N from the

reticulo-rumen. With diets containing large amounts of concentrates and protein in the form of groundnut cake there was evidence of considerable loss of dietary protein (12–54%).

An important approach has been made by OYAERT (1955) who measured the absorption in all three proventriculi together as has already been explained before. In 10 experiments the N-losses varied from about zero to 35%. They were very low on clover hay, intermediate on clover hay plus protein rich concentrate and highest on a diet of clover hay plus casein. In all cases the losses were sensibly reduced by adding 300 gms. potato starch per day to the diet. There was a significant correlation and regression between NH_3-content of the rumen and the N-loss, the regression equation being

$$y = -8.01 + 1.13\,x,$$

where y = percentage of net-nitrogen loss in the proventriculi, and x = mg. NH_3- N per 100 ml. of rumen content.

We already mentioned the techniques used by HOGAN (1957) and HOGAN *et al.* (1960) and that of KAMEOKA *et al.* (1959) who succeeded in avoiding markers by exteriorising the flow of digesta in fistulated sheep. HOGAN *et al.* found a loss of 36% of the digestible crude protein between the mouth and the pylorus, and 64% in the small intestine; for the total crude protein these figures were 25% and 42% respectively.

KAMEOKA *et al.* (1959) collecting in fistulated goats all alimentary tract contents upon entry into the omasum, found on diets of hay and of hay plus concentrates that the quantity of digestible crude protein, digested in the three proventriculi ranged from –20.0 to 52.1%. For the total crude protein these figures were –8.2 to 38.5%. In two of their experiments only grass hay was fed with 11.94% crude protein in the dry matter. In these experiments the loss of digestible crude protein amounted to 0 and –20% which means a gain of + 20%.

The NH_3-absorption in the rumen may be so high that the nutritive value of the food protein sensibly decreases. CUTHBERTSON *et al.* (1950) for instance found that when first class soluble protein (casein) was fed to sheep in need of protein they did not make satisfactory use of it, but if the protein was given straight into the duodenum rather than into the rumen this defective utilization no longer took place. This work has been confirmed and extended by CHALMERS *et al.* (1954a, c), by ANNISON *et al.* (1954) and by HEAD (1953, 1959).

Summarizing, it can be safely stated that large net losses of N may occur in the proventriculi by NH_3-absorption, especially from rations containing a high percentage of protein and little starchy material. Moreover, as a rule, the loss is greater as the proteins used are more soluble; ANNISON's experiments (1956) *in vitro* with bovine albumine suggested however that this is probably not a strict rule.

On the other hand with rations low in protein the net loss was variable. HALE *et al.* and ROGERSON found large losses. The other authors, however, found about a zero-loss or even a gain. In GRAY's experiments with wheaten hay the loss was namely zero; with wheat straw the gain was 17%. BALCH found about zero loss with grass hay, OYAERT found also about zero loss with clover hay (14.4% crude protein in the fresh material), whilst KAMEOKA, in two experiments with grass hay (11.9% of crude protein in the dry matter) stated gains of 0 and 20% of digestible crude protein.

As in our experiments (chapter VII) the protein contents of the rations were generally lower than in those mentioned above, and, moreover, a great deal of starchy material had been added, it is improbable that net N-losses in the proventriculi have occurred. Much more probable is a moderate gain, which might result in high figures for the biological value.

PROTEIN SYNTHESIS IN THE FORE-STOMACHS

The idea that the microorganisms in the fore-stomachs might be profitable to the protein metabolism of the ruminant has been formulated in 1891 by ZUNTZ and HAGEMANN.

ZUNTZ suggested that, for their metabolism, the bacteria would utilize the amides in preference to the proteins so that the latter would be protected from degradation. Although he mentioned the possibility that the bacteria would build up bacterial protein from amides, stress was laid upon the protective action.

Still better in agreement with modern conceptions is HAGEMANN's wording. He reminded of the fact that the microorganisms flourish in a fluid containing amides instead of protein. From the amides they form their body protein, which could be digested later on, so that the animal body protein would be produced from the microorganisms. In case, however, that the amount of amides would be insufficient, they would use the protein which would be degraded and partially lost for nutrition.

The two conceptions are closely related so that they are usually mentioned as the ZUNTZ-HAGEMANN theory.

The theory of ZUNTZ and HAGEMANN has given rise to an immense number of investigations, some of them covering theoretical aspects, most of them with the intention to reduce true protein in practical rations (Reviews: KREBS, 1937; VAN DER MEULEN, 1940). In the Netherlands such investigations with milk cows were performed at the experimental stations at Hoorn and at Hoogland (BROUWER *et al.*, 1943). Relief of protein deficiency by feeding urea or ammonium lactate to dairy cows was, however, only obtained on rations very poor in protein containing 70% of the digestible crude protein recommended by LARS FREDERIKSEN's standard (1931), or less.

Much fundamental work has been done in recent years. LOOSLI *et al.* (1949) showed that in the rumen of lambs fed on semi-synthetic rations almost completely devoid of protein all ten essential amino acids were synthesized from urea. All animals were in positive N-balance. The fact that they continued to gain weight on the urea diet, containing no protein, for over 3 months is further evidence of the formation of amino acids. Other studies were carried out using a purified diet containing glycine as the only source of N. Similar experiments were performed by AGRAWALA *et al.* (1953) and DUNCAN *et al.* (1953) with calves and by OYAERT (1954) with sheep.

For a good understanding of the *BV* it would be very instructive to have figures about the quantities of protein leaving the omasum in microbial form and in the unchanged form. It has already been pointed out that proteins with low solubility are less severely attacked by the rumen organisms than the easily soluble ones. Moreover, a variable proportion of the N in the degraded protein is lost as ammonia. It is also agreed that degradation and resynthesis are surely

not so extensive that the *BV* of the protein in rations with 10–12% of crude protein is nearly always the same, namely about 60 (JOHNSON *et al.*, 1942). Definite figures however for the quantities of protein entering the rumen in the original and in the resynthesized form are far from being established.

Nevertheless there is some information about special types of protein. MCDONALD (1948, b; 1954, a), investigating this question based his experiments on the solubility of zein and the insolubility of microbial protein in aqueous alcohol 80% (v/v). In one of his experiments he also made use of the fact that zein is devoid of lysine contrary to microbial protein. When sheep with fistula in rumen and duodenum were fed a partially purified diet to which zein contributed 94% of the total N, it was found that approximately 40% of the zein was utilized by ruminal microorganisms for the synthesis of their own proteins. In their later experiments with casein MCDONALD *et al.* (1957) took advantage of the phosphorus content of casein and found that at least 90% of this protein was degraded in the rumen and utilized for the synthesis of microbial proteins. Several assumptions had to be made by MCDONALD, so that only preliminary results could be obtained.

However important the work of MCDONALD may be, the problem of protein degradation in ordinary rations remained unsolved. Therefore, some authors have considered whether reference substances in the bacteria or in the feed might be useful. MCDONALD (1954, b) described a method for the estimation of adenine and guanine in preparations of protozoa and bacteria, plant leaves and feeding stuffs as well as digesta. SYNGE (1953) discussed the possibility of using diaminopimelic acid, a constituent of nearly all species of bacteria. WELLER *et al.* (1958) made some use of it as well as of vitamin B_{12} present in bacteria but not in plants. Unfortunately, these methods are far from being satisfactory.

GRAY *et al.* (1953) tried to isolate the microorganisms in the rumen of sheep fed on wheaten hay by centrifugation and claimed a conversion of about half the feed N into microbial protein. In later experiments of the same group of workers (WELLER *et al.*, 1958) the values then obtained were corrected with the results of estimations of diaminopimelic acid and vitamin B_{12}. With sheep, again fed on wheaten hay, they found that from 2 to 24 hours after feeding 63–82% of the total nitrogen was present in the rumen as microbial nitrogen.

From these investigations it is clear that no definite figures have been obtained concerning the microbial protein content in the liquor passing to the abomasum. Nevertheless we are prepared to learn that protein degradation in the rumen may attain several tens of percents. In some cases a great deal of the N resulting from degradation may be lost. In protein-poor rations such as used in our experiments however, there is no net loss of N in the forestomachs or even a gain. In this case the nitrogenous degradation products are completely resynthesized into bacterial protein. We may therefore safely conclude that in our experiments the protein entering the abomasum must have for a large part consisted of microbial protein.

NUTRITIVE VALUE OF MICROBIAL PROTEIN

As in our experiments the feed protein must have been largely converted into microbial protein, it was necessary to consider the feeding value of the latter.

STOKES *et al.* (1946) found that the amino acid pattern of microbial protein

does not differ materially from plant and animal protein represented by wheat and beef liver, and CAMIEN *et al.* (1945) concluded that the amino acid composition of *Lactobacillus fermenti* is similar to that of casein. Nevertheless, it is probable that the amino acid pattern of all bacterial proteins is not identical. KAUFMAN *et al.* (1957) namely found the *BV* of the protein of *Escherichia coli* higher than that of *Lactobacillus arabinosus*, whereas the digestibility of *L. arabinosus* was greater than that of *E. coli*.

Figures for the amino acid pattern of rumen bacteria have been obtained by two different approaches, namely either by the analysis of rumen contents of animals on protein-free diet or by isolating the rumen microorganisms from normally fed animals.

The first approach was made by *determining the amino acids* synthesized in the rumen when the animals were fed on purified diets containing urea or other NPN-compounds as the only source of N (LOOSLI *et al.*, 1949; DUNCAN *et al.*, 1953; OYAERT, 1954).

LOOSLI *et al.* (1949) determined the ten essential amino acids in samples of diets, rumen material, and excreta obtained from 3 sheep and 2 goats fed on purified diets containing urea as the only nitrogen source. The diet was composed of cane sugar 25%, maize starch 42%, cellophane 20%, minerals 5%, lard 4% and urea 4%. The authors concluded that the essential amino acids were synthesized in large amounts in ruminants; the rumen material contained 9–20 times more of the amino acids than the diet fed.

DUNCAN *et al.* (1953) fed fistulated calves on a protein-free diet supplemented with urea. Comparing the amino acid content of the rumen proteins with those obtained from a calf fed on a natural ration, they found good agreement between samples taken after 0 and 6 hours. Although there appeared to be a constant small difference in the arginine, leucine, lysine and methionine content, generally, the values showed good agreement, with the exception of phenylalanine. They also concluded that rumen microorganisms can utilize urea-N to synthesize amino acids.

OYAERT (1954) working with sheep on a protein-free diet supplemented with urea and mono-sodium glutamate, determined the essential amino acids in their rumen contents. He computed the biological value of the microbial protein by the following equation:

$$y = 102 - 0.634\,x,$$

where y is the *BV* and x is the largest deficit percentage of amino acid as compared with egg protein (called by MITCHELL *et al.*, 1946 the "chemical score"). In the same manner he computed the results of other investigators and compared their results with his own.

With the aid of the figures reported by LOOSLI *et al.* (1949), DUNCAN *et al.* (1953) and OYAERT (1954), we calculated the distribution of the essential amino acids in percentages of their sum, with the exception of arginine and glycine. The results are inserted in the first three columns of table 3.

The second approach was performed by *isolating the microorganisms* from rumen contents of normally fed animals and by determining the essential amino acids. As far as we are aware, HOLMES *et al.* (1953) and WELLER (1957) were the only ones who studied the amino acid pattern of microbial protein by this method.

TABLE 3. Essential amino acid composition of rumen contents, bacteria, protozoa and herbage protein (expressed as percentages of the sum of the essential amino acids without arginine and glycine)

Amino acids	Rumen contents on protein-free diets			Rumen bacteria from normally fed animals			Rumen protozoa	Herbage protein
	LOOSLI *et al.* (1949) sheep & goats	DUNCAN *et al.* (1953) calves	OYAERT (1954) sheep	HOLMES *et al.* (1953) sheep "dry fed"	HOLMES *et al.* (1953) sheep "green fed"	WELLER (1957) sheep	WELLER (1957) sheep	WELLER (1957) sheep
Histidine	5	6	4	8	10	4	4	5*
Isoleucine	12	14	17	12	11	15	15	12
Leucine	18	18	18	19	17	18	18	19
Lysine	20	16	10	15	12	17	21	11*
Methionine	6	4	5	5	6	7	5	6*
Phenylalanine	9	10	11	12	11	12	13	15
Threonine	14	14	14	11	10	13	11	13
Tryptophan	2	2	3	5	4	–×	–×	5*
Valine	14	16	17	14	19	16	12	14
Sum of the 9 essential amino acids as percentages of the proteins	27.99	34.41	28.40	35.52	35.21	38.85	45.26	43.18

× Tryptophan was not determined.
* Results from LUGG (1949).

HOLMES *et al.* (1953) determined fifteen amino acids in the protein preparations of each of two rumen samples of sheep fed under "dry" and "green" feed conditions. They found very similar patterns of amino acid distribution for the two samples.

WELLER (1957) separated samples of bacteria and of protozoa from rumen fluids of sheep which had been fed on 4 types of rations. The first ration was wheaten hay chaff fed *ad libitum*, the second ration was lucerne hay chaff fed *ad libitum*. These rations contained 0.89 and 2.91 % N respectively. The third ration was composed of wheaten straw (600 gms.) containing 2.3 gm. N daily, crushed oat grain (200 gms.) containing 2.9 gm. N daily and 15 gms. urea providing 7.0 gm. N daily. The fourth ration was mixed green pasture. WELLER determined the amino acids by the ion-exchange chromatography of hydrolysates of "whole protein" preparations of the microbial fractions. He concluded that the compositions of the bacterial hydrolysates were markedly uniform and the amino acid distribution was similar to those reported for pasture leaf proteins. He also reported that protozoa are richer in the essential amino acids than bacteria, particularly lysine.

From the results of HOLMES *et al.* (1953) we also calculated the distribution of the essential amino acids as percentages of their sum (without arginine and glycine). The same was done with the figures of WELLER (1957) referring to the normal rations no. 2 (lucerne hay chaff) and no. 4 (pasture). The results are presented in columns 4–8 of table 3.

We have distinguished sharply between the two approaches because both have their *advantages and disadvantages*. On protein-free diets supplemented with

urea as used in the first approach, the microbial rumen population is probably not the same as on normal diets. Moreover, it is not certain that on such diets all the microbial protein-N originates from urea-N. There also seems to be a possibility that amino acids from the saliva or even from the blood, after passing the rumen walls, are ulitized by the rumen microorganisms for building up their body protein.

The second approach consisting in the isolation of bacteria from normal rumen contents and in the determination of their amino acid pattern is also open to criticism. As a matter of fact the samples of isolated bacteria are never representative of the total rumen population because only the larger microorganisms can be secured by centrifugation. Moreover, the separation of microorganisms and small food particles is far from complete.

Nevertheless, it appears from table 3 that the results of both approaches are essentially identical. Furthermore, there are also only small differences between the bacterial proteins on the one hand and the protozoal protein and herbage protein on the other. It should however be pointed out that the protozoa show an exceptional high lysine content.

In comparing table 3 with table 2 presenting figures for amino acid requirements of men and animals it appears that there is a fairly good agreement. The methionine content in the microbial and herbage proteins is lower than required for maintenance of rats and men. It should however be taken into consideration that cystine may supplement the methionine.

In the bottom line of table 3 the sum of the nine essential amino acids are given as percentages of the proteins in which they partake. The sums referring to the bacterial proteins are somewhat lower than those referring to the protozoal and herbage proteins and, also, as compared with the sum in whole egg protein (49.6) (OSER, 1951). Nevertheless a fair quantity of the bacterial proteins consists of essential amino acids.

It must therefore be concluded that the proteins of the rumen microorganisms are well-balanced and of high grade, containing the essential amino acids in favourable proportions and in reasonable amounts. Dietary proteins deficient in certain amino acids and even non-protein-nitrogen-compounds may be transformed by the rumen microorganisms into proteins with highly satisfactory amino acid patterns.

The feeding value of a protein however is not accurately defined by its amino acid pattern. The *digestibility* is also important and, moreover, the *biological value* cannot exactly be predicted from the amino acid composition, but should be checked by experiments with test animals.

It is probable that the digestibility and the biological value of the microorganisms entering the abomasum of the ruminant can be measured with reasonable accuracy with rats as test animals. Several efforts have been made in order to isolate the rumen microorganisms in quantities large enough to perform such tests. The best method seems to be differential centrifugation of rumen contents. Although in this manner some results have been obtained, so far nobody has succeeded in isolating a protein perfectly representative of the microbial population in the rumen.

REED *et al.* (1949) prepared two large samples of "mixed" rumen bacteria, virtually free from protozoa, from rumen contents of abattoir sheep, one sample from sheep coming from "green" feed conditions and the other from "dry"-fed

sheep. MCNAUGHT *et al.* (1950, 1954) incubated rumen liquid from cows with maltose or glucose before isolating the bacteria. By this incubation the amount of bacterial protein increased by 34%. Protozoa were obtained without incubation. Both groups of authors tested the bacteria obtained in rat tests with the following results (four upper lines).

		Bacterial protein	
		True digestion coefficient	*BV*
REED *et al.* (1949)	Sheep, green fed	62	80
	Sheep, dry fed	65	78
MCNAUGHT *et al.* (1950)	Cows	73	88
MCNAUGHT *et al.* (1954)	Cows	74	81
KAUFMAN *et al.* (1957)	*E. coli* untreated . . .	81	69
	L. arabinosus „	84	52
	E. coli dried	83	66
	L. arabinosus „	89	56

The results indicate that the digestibility of the bacterial protein is moderate whilst the biological value is rather high, though not as high as that of the best animal proteins. These figures are somewhat different from those of KAUFMAN *et al.* (1957) with pure cultures of *E. coli* and *L. arabinosus* (four lower lines). It is not clear whether the lower digestibility and the higher *BV* of the proteins in the rumen bacteria may be due to admixture of feed protein or to specificity of bacteria.

The digestibility of the protozoal protein was examined by MCNAUGHT *et al.* (1954) and found to be 91, thus considerably higher than that of the bacterial protein; the biological values of protozoal and bacterial protein were about the same, 80 and 81 respectively.

In concluding this chapter it can be stated that the conversion of herbage protein to microbial protein will not bring about a marked decrease in nutritive value, if there are no net losses of NH_3 by absorption. In our experiments with low protein diets such losses are very unlikely. It is even more probable that there was a net gain of protein-N originating from salivary urea-N and, as a result, a net gain in protein value.

CHAPTER III

METABOLIC FAECAL NITROGEN (PROTEIN)

INTRODUCTION

Since the investigations of RUBNER (1879), HERMANN (1889) and others it is known that in the intestine not only absorption takes place but also excretion. It is obvious therefore that the faeces do not only contain residues from the feed, but also products originating from the intestine and the intestinal glands. This has been shown by giving a protein-free food to rats, pigs, horses and sheep for many days. In these experiments the faeces do not become devoid of protein

but there always remains a considerable percentage of protein in the excreted material. This originates from bile, pancreatic juice, gastric and intestinal secretions, abraded epithelium cells and microorganisms that have developed in the intestinal contents.

Many years ago, already the German investigators were very much interested in these "Stoffwechselprodukte" (products of metabolism), because the knowledge of the quantities of these products is absolutely necessary for calculating the true digestion coefficients from the apparent digestion coefficients as determined in the ordinary way, by subtracting total faecal crude protein from feed crude protein. The true digestibility is mainly determined by the feed constituents absorbed from the intestinal tract into the blood stream. The true digestion coefficient D for instance of nitrogen, is $100\,\frac{A}{I}$, where A is absorbed nitrogen and I is N-intake. A is calculated as follows: $A = I - (F - Fx)$ or N-intake minus faecal N from food, where F is the total faecal N and Fx is the excretion of N in faeces originating from the body and not from food sources, the so called metabolic faecal nitrogen (*MFN*) or metabolic faecal protein (*MFP*).

It should however be kept in mind that the above mentioned question for many years was mainly a theoretical one because in practical feeding not the true digestion coefficients are used, but the apparent ones.

New interest in the metabolic faecal protein has risen after the introduction by THOMAS (1909) and MITCHELL (1924) of their method for calculating the biological value of protein. As a matter of fact, the metabolic faecal protein (*MFP*) is an essential element in the formula of THOMAS and MITCHELL for calculating the biological value.

Unfortunately, the experimental determination of the *MFP* in sheep and other ruminants is not nearly as easy as in rats and pigs. This was the reason why, just like many other authors, we did not succeed in estimating the *MFP* directly in faeces from animals fed on completely protein-free diets, but had to have recourse to *indirect* methods. With this aim in view the literature on this subject was scrutinized.

A. DIRECT METHODS

1. *Digestion in vitro*

As the older investigations refer to ruminants, it is conceivable that in earlier days not the direct methods have been developed, but, on the contrary, the indirect ones.

After STUTZER had introduced the artificial digestion with gastric juice in feed analysis, KELLNER (1880) applied this method to several feeding stuffs whose digestibility had formerly been determined in experiments with animals. In this way it was shown that the undigested protein residues in natural digestion were larger than those in artificial digestion. It seemed probable to KELLNER that the difference should be attributed to the "Stickstoffhaltige Stoffwechselprodukte". From his observations he concluded that these products amounted to 0.3–0.5, with an average of 0.4 parts of nitrogen, or 2.5 parts of protein per 100 parts of digested dry matter. These findings were essentially confirmed by PFEIFER and KÜHN and several other authors. Taking into account all investigations KELLNER concluded that the average is 0.45 gm. of N or 2.8 gm. of crude protein per 100 gms. of digested dry matter.

MORGEN *et al.* (1914), however, showed that the problem is more complicated. They found that the *MFP* varies considerably with the composition of the feed. Moreover, the treatment with pepsin-HCl fails to dissolve the total quantity of the "Stickstoffhaltige Stoffwechselprodukte". It was their opinion that after the treatment with pepsin-HCl, a treatment with trypsin was necessary. Even in this way the solution of the "Stoffwechselprodukte" probably was not yet completed. After KRZYWANEK (1929) the pepsin-HCl values were about $^1/_3$ lower than the true values.

On the other hand, it should be kept in mind that the *MFP* does not derive from intestinal secretions only. The constituents soluble by pepsin-HCl comprise an unknown part of the proteins in the microorganisms. These proteins may derive from intestinal secretions as well as from food proteins.

This argument seems to be at variance with the conclusion of KELLNER (based on experiments in which asparagine and ammonium acetate were fed) that the existence of great quantities of bodies of bacteria in the faeces is more than questionable. This conclusion has however been challenged by HUISMAN (1946).

Newer investigations have shown that the conversions in the rumen are far more extensive than formerly was believed (chapter II). Perhaps the same applies to the conversions in the caecum and colon. In the rumen presumably about half of the feed protein is converted into bacterial protein. It is by no means certain that all this bacterial protein is digested and absorbed in the lower intestine. As a matter of fact the faecal protein consists for a large part of microbial protein and it is not known how much of this microbial protein originally comes from food protein or from secretory protein. It is highly probable that in artificial digestion of faeces with pepsin-HCl all secretory protein and a large part of the bacterial protein is dissolved. It seems, therefore, that with pepsin-HCl we determine not only the protein in the secretory products but also part of the degraded proteins that have failed to be absorbed and have been synthesized to bacterial protein. Thus, for this reason, the figure for *MFN* may be too high. However we have already mentioned another reason for which the figure may be too low (MORGEN *et al.*, 1914; KRZYWANEK, 1929). It is therefore clear that the *MFN* in ruminants deserves closer examination in the near future.

This uncertainty, at first sight, seems to be very serious to the accuracy of the calculation of the biological value (*BV*). Fortunately, it has been shown that the *MFN*, although entering in the formula of *BV*, does affect the results only slightly. In other words, the figure for biological value is not very sensitive to inaccuracies in the *MFN*.

For further details on the questions concerning the method of digestion *in vitro* we refer to the above mentioned paper of HUISMAN (1946). We only add that according to KRZYWANEK (1929) in the artificial digestion only fresh faeces should be used, because in drying, even at low temperatures, there is a decrease of the digestibility amounting to about 6%. Drying would be permitted only when special precautions are taken.

Another objection to KELLNER's conclusion is that he related the *MFP* to the *digested* dry matter. Supposing that the digestion coefficient of the dry matter is c and the dry matter intake is D, then the quantity of metabolic faecal nitrogen M is

$$M = 0.028\ c\ D.$$

Other authors have shown that for equal intakes of dry matter the metabolic faecal nitrogen is independent of or even increases with the crude fibre content of the ration and, therefore, also with the percentage of indigestible dry matter. This means that *M* remains the same or even increases with a lower digestion coefficient *c*. From the formula, however, it would follow that *M* would decrease with lower digestion coefficient *c*.

Although the accuracy of the underlying determinations has been challenged and the activity of the microflora has not been properly taken into consideration by the older investigations, undoubtedly some information may be obtained. It is however not advisable to calculate *MFP* by making use of KELLNER's formula. Later on we shall mention another formula based on results of experiments with sheep on a protein-free diet. Nevertheless, we also considered it worth while to estimate in every instance the *MFP* by artificial digestion of the faeces with pepsin-HCl.

2. *Direct determinations*

Direct determination of metabolic faecal protein with animals on diets containing no or very little nitrogen gives very good results in non-ruminants such as rats and pigs. They eagerly eat such rations for a couple of weeks and their health is not sensibly disturbed.

As already has been mentioned, with ruminants the direct determination of *MFN* presents many difficulties. Usually the animals eat the protein-free food only reluctantly and it often happens that after a few days appetite decreases and considerable residues are left in the feed boxes.

Also from a theoretical point of view the determination of *MFN* with ruminants on protein-free diets is not sound. It is clear that on protein-free diets the rumen bacteria develop insufficiently so that breakdown of cellulose is slowing down and the conversion of the other carbohydrates into volatile fatty acids and other degradation products is incomplete. The result is that more dry matter enters the abomasum and small intestine, and the digestion shifts to the type as occurring in non-ruminants.

This was already known to the earlier German investigators. They stated a decreased digestion, "Verdauungsdepression", in diets with wide nutritive ratios. This was particularly true for protein, but here the effect can be readily explained by the output of the metabolic nitrogen, since the protein digestibility represents the apparent digestibility, and it has been definitely shown that the apparent digestibility of protein is lowered with a wide ratio even though the true value may be not. This is also shown by an extensive statistical study of HUISMAN (1946) which will be discussed later on. Thus, the "Verdauungsdepression" of protein mainly is an apparent one. Moreover, the depression of food protein digestibility of course is immaterial with protein-free diets.

It has however been shown that the N-free extractives and the crude fibre also may suffer "Verdauungsdepression" in diets with wide nutritive ratio. As the intestinal products of metabolism hardly contain any carbohydrate and no crude fibre at all, it is clear that with these substances the lowering of digestibility must not be an apparent but a real one. From all this it results that digestion of protein-free feed by ruminants is abnormal so that reliable values for *MFN* may be hardly expected.

Nevertheless, it is worth while to discuss the results obtained by a few in-

vestigators who succeeded in conducting experiments with ruminants (sheep) on protein-free or nearly protein-free diets. In table 4 we present these diets fed to sheep which were collected from the literature.

TABLE 4. Low nitrogen diets fed to sheep, collected from the literature

Author / Food-stuffs (%)	SOTOLA (1930)	SOTOLA (1933)	TURK *et al.* (1934)	MILLER (1937)	SMUTS *et al.* (1938)	HARRIS *et al.* (1941)	MILLER *et al.* (1942)
Agar	–	–	–	–	20.0	–	–
Cellulose	–	–	5.0	18.0	–	–	10.5
Citric acid	–	–	–	–	–	0.58	–
Oil	1.5	1.5	4.5	4.0	2.0	4.50	4.5
Salt	–	–	3.5	4.5	5.0	2.00	4.0
Starch	31.0	25.7	23.5	53.5	73.0	28.42	28.0
Straw	51.5	47.1	40.0	–	–	25.00	25.0
Sugar	16.0	25.7	23.5	15.0	–	29.00	28.0
Wood pulp	–	–	–	–	–	10.50	–
Yeast	–	–	–	5.0	–	–	–

In table 5 we find some results for the *MFN* obtained on the nearly protein-free diets.

TABLE 5. The metabolic faecal nitrogen determined with sheep on protein-free diets as found in the literature

Name of author	Year	Kind of animal	Metabolic faecal nitrogen gm./100 gms. of dry matter intake
MORGEN *et al.* (quoted by TURK *et al.*)	1914	sheep	0.5100
SOTOLA	1930	lambs	0.6500
TURK *et al.*	1934	lambs	0.5600
HUTCHINSON *et al.*	1936	sheep	0.4500
MILLER *et al.*	1939	lambs	0.5500
HARRIS *et al.*	1941	,,	0.5550
HAMILTON *et al.*	1948	,,	0.5563
Average	–	–	0.5473 (corresponding to 3.438 gm. protein)

In these experiments considerable difficulties were met which make the results unreliable. The animals under investigation showed symptoms of extreme discomfort, loss of appetite and weight, and the digestive processes were badly upset. The consumption of feed decreased and sometimes the animals went off feed altogether. Moreover, the duration of these experiments was not long enough to give reliable figures.

In rats and pigs, however, the method has proved to be very valuable. MITCHELL *et al.* (1954), for instance, after testing its validity on growing rats, and after surveying similar tests from other laboratories judged the direct measurement beyond reasonable doubt to be valid.

B. INDIRECT METHODS

1. *Protein-free diets with the addition of a small quantity of highly digestible protein*

This method comes close to the direct one. The added protein might, for instance, be egg white protein or milk protein. The true digestibility of these proteins is nearly 100%. If desired it can be determined in a separate digestibility experiment or an appropriate figure may be taken from the literature. By subtracting the calculated faecal residue from the total faecal protein, the *MFP* is found. MITCHELL *et al.* (1926) were the first who employed this method and proposed to substitute a low-egg N ration for an N-free ration in protein studies with rats. They found that the excretion of faecal N per gm. of food consumed and of urinary N per 100 gms. of body weight by the rat was practically the same as on a protein-free ration. As far as we know until now this method has not been used in experiments with ruminants.

2. *Extrapolation methods*

This method was introduced by TITUS (1927) in determining the true digestibility of lucerne protein with steers. He varied the protein content of the ration while keeping the total dry matter intake constant and plotted the faecal N excretion against the total protein intake. The data were then fitted by a regression line and extrapolated to the point zero N intake; the y intercept was used as the *MFN* excretion.

It is evident that essentially the same value must be obtained if not total crude protein but digestible crude protein is plotted on the abscissa.

The mathematics of this method is very simple. Suppose in two experiments with equal dry matter intake the quantity of the protein examined is successively X_1 and X_2 gms. and the total faecal protein y_1 and y_2 gms. Then the equation for the straight line may be given by either the following two equations:

$$Y - y_1 = (X - x_1)\frac{y_2 - y_1}{x_2 - x_1},$$

$$Y - y_2 = (X - x_2)\frac{y_2 - y_1}{x_2 - x_1}.$$

From this, we easily find

$$Y - \tfrac{1}{2}(y_2 + y_1) = \left\{X - \tfrac{1}{2}(x_2 + x_1)\right\}\frac{y_2 - y_1}{x_2 - x_1}.$$

By assuming that

$$\tfrac{1}{2}(x_2 + x_1) = U_x, \qquad \tfrac{1}{2}(y_2 + y_1) = U_y,$$
$$\tfrac{1}{2}(x_2 - x_1) = V_x, \qquad \tfrac{1}{2}(y_2 - y_1) = V_y,$$

we obtain

$$Y - U_y = (X - U_x)\frac{V_y}{V_x}.$$

The metabolic faecal protein Y_0 follows when $X = 0$. Thus,

$$Y_0 = U_y - U_x\frac{V_y}{V_x}.$$

It is evident that the standard errors of U_x and U_y are relatively small in comparison with those of V_x and V_y. Again, the standard error of V_x is small in comparison with that of V_y. Calling σ the standard deviation of V_y and neglecting the standard deviation of U_x, U_y and V_x, we find for the standard deviation of Y_0:

$$^{\sigma}Y_0 = \frac{U_x}{V_x}\,\sigma.$$

As V_x is ordinarily small in comparison with U_x it is apparent that $^{\sigma}Y_0$ is essentially dependent on σ and $V_x = \frac{1}{2}(x_2 - x_1)$. The latter means that it is advantageous to make $x_2 - x_1$ as large as possible.

In deriving these formulae it is supposed that the dry matter intake and the crude fibre content are the same in the two rations. In case this might not be true corrections may be necessary.

3. *Statistical methods*

HUISMAN (1946) tried to calculate the amount of *MFP*, basing himself on data taken from the literature on digestible nutrients in a large number of feeding stuffs. He calculated the regression of apparent digestible crude protein (y) on the total crude protein (x), both on organic matter basis. In this way he found:

$$\left.\begin{array}{l}\text{for grass} \quad y = 0.939\,x - 3.46,\\ \text{for hay} \quad y = 0.837\,x - 3.13, \text{ and}\\ \text{for foodstuffs of vegetable origin from KELLNER's tables}\\ \qquad y = 0.923\,x - 3.90\end{array}\right\} \qquad (1)$$

By extrapolation to $x = 0$ the metabolic faecal protein is obtained, successively amounting to 3.46%, 3.13% and 3.90% of the ingested organic matter.

In the literature it is suggested that the *MFP* might be positively correlated with the crude fibre. Therefore, he calculated the multiple regression of y (apparent digestible crude protein) on x (total crude protein) and z (total crude fibre), all three on organic matter basis. The results were as follows:

$$\left.\begin{array}{l}\text{for grass} \quad y = 0.946\,x + 0.010\,z - 3.83,\\ \text{for hay} \quad y = 0.829\,x - 0.032\,z - 1.90,\\ \text{and for foodstuffs of vegetable origin}\\ \text{from KELLNER's tables}\\ \qquad y = 0.905\,x - 0.040\,z - 2.58.\end{array}\right\} \qquad (2)$$

As the coefficients of z are very small he concluded that the crude fibre has only a small influence on protein digestibility and on *MFP*. With a crude fibre content of about 26% of the dry matter as occurring in the hays used in our experiments, the *MFP* would be successively 3.53, 2.86 and 3.78 gms. for 100 gms. ingested organic matter.

Most figures published in the literature do not refer to organic matter basis but to dry matter basis. The above mentioned equations (1) can, however, easily be transformed to dry matter basis.

Suppose p = organic matter (%) in dry matter of feed,
x' = crude protein (%) in dry matter of feed, and
y' = digestible crude protein (%) in dry matter of feed.

Then $x = \frac{100}{p} x'$ and $y = \frac{100}{p} y'$.

If $p = 90.5$ as occurred in our hays, it follows that

$$x = 1.105\, x' \quad \text{and} \quad y = 1.105\, y'.$$

Substitution in (1) gives

for grass $y' = 0.939\, x' - 3.13$,
for hay $y' = 0.837\, x' - 2.83$,
for foodstuffs of vegetable origin from KELLNER's tables $y' = 0.923\, x' - 3.53$.

Thus, with 90.5% of organic matter in the dry substances the results are successively 3.13, 2.83 and 3.53 gms. of *MFP* per 100 gms. of dry matter intake.

Additional results may be taken from the papers of AXELSSON (1941, 1942). By calculating, from experiments with horses, the regression of crude protein in the faeces on crude fibre in the feed, he practically found a zero regression coefficient. By analysis of experiments with ruminants he even found a small negative correlation, but this seemed to be caused by the fact that the crude protein content in the feed decreased a little as the crude fibre content rose. Calling x = gms. of crude protein per 100 gms. of dry matter in the feed, and v = gms. of crude protein in the faeces per 100 gms. of ingested dry matter, he found that $v = 0.0073\, x + 3.4$.

Thus, with a protein-free feed ($x = 0$), the crude protein content in the faeces (which means the *MFP*) is 3.4 gm. per 100 gms. of ingested dry matter, which is in good agreement with the findings of HUISMAN (1946).

BLAXTER *et al.* (1948), apparently not aware of the work of BROUWER *et al.* (1938) and DIJKSTRA *et al.* (1939), to be mentioned later on, and of that of HUISMAN (1946), used the same formulae for calculating the regression of digestible protein on total protein in the dry matter of some classes of foodstuffs and came to the following formulae:

European grass hays	$y' = 0.788\, x' - 2.94$,
Lucerne hays	$y' = 0.881\, x' - 2.86$,
Pasture grass	$y' = 0.931\, x' - 2.73$,
Cereal straws	$y' = 0.672\, x' - 1.67$,
Root crops	$y' = 0.907\, x' - 2.37$,
Silages	$y' = 0.800\, x' - 1.97$,
Oil seed cakes and meals	$y' = 0.914\, x' - 1.84$.

Taking into account only the first three equations with constants 2.94, 2.86 and 2.73, and computing the average, they concluded that the weighted mean value for *MFP* is 2.811 ± 0.676 per 100 gms. of dry matter ingested.

If there were in ruminants a distinct positive partial correlation between crude fibre and *MFP*, the value for cereal straws would have been considerably higher than 2.811. On the contrary, this value was lower, namely 1.673 ± 0.401. The authors suggested that this might be due to possible elimination of negative digestion coefficients or to the conversion from negative values to zero when compiling the tables from which the data were abstracted. At any rate, the formulae of BLAXTER *et al.* mentioned above do not suggest a positive correlation between crude fibre content and metabolic faecal protein.

This argument cannot be abolished by the fact that for oil seed cake meals which were poor in crude fibre also a low value (1.840 ± 1.637) was found, although not as low as that for the cereal straws. The standard deviation of this figure is however four times as large as that derived from the cereal straws. The authors are undoubtedly partially right in saying that this is merely a reflection of the greater error of extrapolation to the $x' = 0$ intercept in the case of feeds of high protein content.

In our opinion, a second factor increasing the variability of the results should not be overlooked. This consists in the fact that, in determining the digestibility, the oil cake meals cannot be fed as a single feed but only as an addition to a roughage-containing basal ration, the digestibility of which must be determined in a separate experiment. It is obvious that this reduces the accuracy of this "indirect" method of digestibility determination.

It is even justified to raise the question whether the regression equations obtained from indirect digestibility experiments are on a line with those from direct ones, and whether it is allowed to identify the constant term in the "indirect" equations with the *MFP*. HUISMAN, however, payed due attention to this point and came to the conclusion that in this respect at least, no objections can be made, provided that there is no mutual influence of the feeds.

The second formula (b) of HUISMAN referring to hay is derived from 142 samples originating from six different sources. BROUWER *et al.* (1938) and DIJKSTRA *et al.* (1939) calculated the regression equation for each of these six sets separately and arrived at the following formulae (organic matter basis):

$$y = 0.665\,x + 1.77,$$
$$y = 0.824\,x + 2.95,$$
$$y = 0.832\,x + 2.85,$$
$$y = 0.833\,x + 3.74,$$
$$y = 0.765\,x + 2.43,$$
$$y = 0.746\,x + 1.66.$$

The weighted average was:

$$y = 0.785\,x + 2.47.$$

The weighted average of the constant term is therefore 2.47, whereas in the fresh grass HUISMAN found 3.46, as already has been mentioned. Just as in the paper of BLAXTER *et al.* the absolute value of the constant term for hay is lower than that for fresh grass. Whether the difference is a real one is not quite sure. Elimination of negative digestion coefficients in hays can hardly be feared. There remains however the small possibility that the temperature of fermentation in the hays might have induced a somewhat greater decrease of protein digestibility.

4. *Isotope methods*

A new technique using isotopes has been devised by LOFGREEN *et al.* (1953) for determining metabolic faecal nitrogen in calves fed on purified liquid diets. They stated that their method is based on three assumptions:

1) that the liberation of P from casein is proportional to the digestion of this protein,

2) that the protein of the casein which passes through the tract undigested has the same $N:P^{32}$ ratio as that which was fed, and

3) that all the isotope appearing in the faeces comes directly from the undigested casein.

A correction can be made for a small amount of P^{32} which has been absorbed and reexcreted.

Obviously, the meaning of the third assumption is that all the P^{32} in the faeces is contained in undigested casein. For this assumption, however, no definite arguments have been put forward. On the contrary, we believe that part of the P in the faeces is present in inorganic compounds such as calcium phosphates, although this is perhaps more the case in adult cattle than in very young calves. This might be the reason or one of the reasons why the values of LOFGREEN *et al.* for *MFP* (0.27 gm. N or 1.69 gm. protein) per 100 gms. of dry matter consumed, even with young calves, are sensibly lower than those of BLAXTER *et al.* (1951) (0.43 gm. N or 2.69 gm. protein per 100 gms. of dry matter ingested). However, leaving the question as it is for young calves, it seems to us that for older cattle or sheep the results of this method must be unreliable.

C. FACTORS INFLUENCING THE AMOUNT OF METABOLIC FAECAL PROTEIN

MITCHELL *et al.* (1954) concluded that the direct measurement of *MFN* in the determination of the *BV* of protein is more economical in time and animals than the method involving the establishment of a regression equation between faecal N and N intake. In our opinion this only refers to animals such as rats and pigs, and not to ruminants, owing to the difficulties experienced with these animals on protein-free rations, as explained in paragraph B of this chapter.

KELLNER related the *MFN* to the digestible dry matter intake. The younger authors determined the correlation with total dry matter intake. SMUTS *et al.* (1938), for instance, working with sheep, plotted the *MFN* for two N-free periods against the corresponding quantities of dry matter intake and found a strong positive correlation between these values.

While it seems that the most advisable way of expressing the *MFN* is in terms of dry matter intake, MILLER *et al.* (1942) stated from their experiments with lambs that body weight and possibly other factors may have some influence. SCHNEIDER (1934) working with rats, divided the *MFN* into two fractions: first, a true secretory fraction that continues at a constant level, even during fasting, and which may be related to the basal nitrogen metabolism; and secondly, a fraction that is secreted as a result of the ingestion of food and in amounts proportional to the dry matter consumed.

Not always the *MFN* was related to the dry matter intake; ELLIS *et al.* (1956), for example, expressed the *MFN* as milligrams per gm. of faecal dry matter excreted. They found that it was less variable when expressed on this basis than when expressed on the basis of dry matter intake and they reported the results for sheep to be in accordance with observations on men and cattle.

The influence of crude fibre on *MFN* has been discussed several times. MITCHELL (1943) showed that the *MFN* is not only proportional to the dry matter of the diet but also to the roughage content of the ration. For animals that consume low-roughage diets such as rat, dog, pig and man, an arbitrary figure of 0.22 gm. N (1.375 gm. protein) per 100 gms. ration is often used as value for the *MFN*. In case of cattle and sheep the rations of which ordinarily

contain higher amounts of roughage, the value of 0.55 gm. N (3.438 gm. protein) is employed.

MORGEN *et al.* (1914) mentioned that, in pigs, rations with much starch or crude fibre gave the highest values for *MFN*. The average in sheep was 0.85 gm. N or 5.3 gm. crude protein per 100 gms. of digestible organic matter, in pigs it was 0.4 gm. N or 2.5 gm. protein. In sheep, rations with a high content of crude fibre containing incrustating substances would increase the *MFN* values. It should however be mentioned that MORGEN *et al.* related the *MFN*-values to *digested* organic matter. MORGEN's values are therefore not necessarily at variance with the results of the statistical analyses by HUISMAN (1946), AXELSSON (1941, 1942) and BLAXTER *et al.* (1948) who related the *MFN* to either *organic* or *dry matter* and found little rise in *MFN* with increasing crude fibre content of the feed (§ B of this chapter).

TITUS (1927) concluded that the amount of metabolic N in the faeces of a steer is influenced, among other things, by the water content of the faeces. When this water content was corrected to a uniform level of 80%, a linear relationship existed between the N content of the faeces and the N content of the feed, especially when the feed N was decreased by substituting paper pulp for an equal weight of a given ration of lucerne. He was already of the opinion that the faecal N excretion of a steer consuming an N-free ration cannot safely be taken as a measure of the *MFN* of the animal when consuming an equal weight of a given feeding stuff.

D. COMPARISON OF THE RESULTS OBTAINED BY THE DIFFERENT METHODS

In the preceding discussion of this chapter the various methods used in determining the metabolic faecal nitrogen (protein) have been reviewed. We found that figures obtained by employing the various techniques varied a great deal. The lowest value was that secured by LOFGREEN *et al.* (1953) who used the isotope method. A figure of 0.27 gm. N (1.69 gm. protein) per 100 gms. of dry matter consumed was reported with a calf on a liquid diet. All the other authors gave values for the *MFP* per 100 gms. of dry matter intake with ruminants which were much higher than those obtained by LOFGREEN *et al.* These values in ascending order are: 2.12 (TITUS, 1927 as cited by HUTCHINSON *et al.*, 1936), 2.24 (BROUWER *et al.*, 1938), 2.83 (HUISMAN, 1946), 2.90 (BLAXTER *et al.*, 1948), 3.4 (AXELSSON, 1941, 1942) and 3.438 (on protein-free feed, as collected from the literature and reported in this chapter, table 5).

We have chosen the latter figure for calculating the amount of *MFP* and of the *BV* in our experiments because it has been obtained experimentally by actually feeding N-free rations to sheep. Moreover, the extrapolation and statistical methods (TITUS, 1927; BROUWER *et al.*, 1938; HUISMAN, 1946; BLAXTER *et al.*, 1948; AXELSSON, 1941, 1942) yielded figures not far from those secured experimentally with sheep on the N-free diets.

As already mentioned in paragraph C of this chapter, some authors have stated that not only the dry matter intake but also other factors may affect the *MFN*-values. As a matter of fact nearly all authors take only the dry matter intake into account. We have followed this rule, considering that the influence of the other factors has been studied insufficiently and that presumably little would be gained in accuracy, the more so as the whole question of *MFN* in ruminants is somewhat vague.

This is also the reason why in our experiments we have not only confined ourselves to calculations of the amounts of *MFN* from the dry matter intake, but, in addition, we have made determinations of metabolic faecal protein with pepsin-HCl.

CHAPTER IV

ENDOGENOUS NITROGEN (PROTEIN)

In assessing the biological value of proteins, it is necessary to know how much of the nitrogen absorbed from the alimentary tract is retained in the body. This requires the measurement of faecal-N and the urinary-N as well.

FOLIN (1905) drew attention to the fact that the excretion of urea and some other urinary constituents vary a great deal according to the amount of protein ingested, whilst the quantity of some other metabolism products such as creatinine remains nearly constant, even when the protein in the food is diminished so far that no protein is furnished at all. From these facts FOLIN concluded that there must be two kinds of protein catabolism, essentially independent and quite different, and partly with two different sets of waste products.

It was clear to FOLIN that the metabolic processes resulting in the end products which tend to be constant in quantity appear to be indispensable for the continuation of life and probably constitute an essential part of the activity which distinguishes living cells from dead ones. This protein metabolism tending to be constant was called by him the tissue metabolism or *endogenous metabolism.* The other, the variable protein metabolism, consisting of a preliminary removal of unnecessary nitrogen by means of hydrolytic splitting and resulting in the excretion of urea was called the *exogenous metabolism.*

Assuming that the endogenous nitrogen excretion is independent of the nitrogen intake it is evident that its quantity can be determined on a protein-free ration. It should, however, be stressed that the diet must be adequate in other respects, because by shortage of energy (starch value) the organism attacks not only its own fat-reserves but also a liberal amount of body protein in addition to the amounts broken down in endogenous catabolism.

This theory of FOLIN has been discussed many times (MCCOLLUM *et al.*, 1913; OSBORNE *et al.*, 1919; BORSOOK *et al.*, 1935; MITCHELL, 1943). The most serious objections were brought forward by SCHOENHEIMER *et al.* Using isotopes to label the amino acids whose metabolism was studied, SCHOENHEIMER *et al.* (1939) found extensive interchange of nitrogen between dietary amino acids and tissue protein. They showed that nitrogenous groupings of tissue proteins are constantly involved in chemical reactions; peptide linkages open, the amino acids liberated, mix with others of the same species of whatever source, diet or tissue. This mixture of amino acid molecules, while in the free state, takes part in a variety of chemical reactions; some re-enter directly into vacant positions left open by the rupture of peptide linkages; others transfer their nitrogen to de-aminated molecules to form new amino acids. These in turn continuously enter the same chemical cycles which render the source of nitrogen indistinguishable. Some body constituents like glutamic and aspartic acids and some proteins like those of liver, serum, and other organs are more actively involved than others in this general metabolic mixing process. The excreted nitrogen may be considered as a part of the metabolic pool originating from

interaction of dietary nitrogen with the relatively large quantities of reactive tissue nitrogen.

From these facts they concluded that it scarcely would be possible to reconcile their findings with any theory which requires a distinction between endogenous and exogenous nitrogen.

It appeared therefore that "exogenous and endogenous metabolites are pooled in the living organism" (BIGWOOD, 1952) so that it seemed that FOLIN's theory had to be dropped. Especially one circumstance however has prevented it from being swept away altogether. Practically all investigations namely, performed after FOLIN's paper (1905), confirmed the constancy of creatinine excretion, independent of higher or lower protein contents of the feed. The literature on this subject is very extensive as far as men and laboratory animals are concerned. On the contrary, the papers referring to ruminants are very scarce. Nevertheless they are in agreement with the results obtained with other animals and with men. DINNING *et al.* (1949), for instance, concluded that the daily excretion of creatinine nitrogen by beef steers is relatively constant and is unaffected by changes in the protein content of the ration. From experiments in the Laboratory of Animal Physiology at Wageningen, DE GROOT and AAFJES (1960), working with dairy cows, also found that creatinine was excreted in the urine at a constant rate during day and night, and that it could be used as a reliable measure for the secretion of urine constituents. This constancy did not hold for the quantity of creatine or that of creatine plus creatinine. They proposed that the concentration of urinary constituents should be expressed in terms of creatinine quotient rather than in absolute units as is done in veterinary practice.

Although the opinion that creatinine is excreted at a rather constant rate remained undisturbed, the interpretation of this fact has changed a good deal in the course of time. FOLIN, as already mentioned, regarded creatinine as "an index or measure of the total normal tissue metabolism", and probably as "an essential part of the activity which distinguishes living cells from dead ones". SHAFFER (1908–9) however, suggested that creatinine is not derived from, and an index of, the total tissue or endogenous catabolism, but of one special process of catabolism, taking place largely if not wholly in the muscles, upon the intensity of which depends the muscular efficiency of the individual. Others have assumed that this excretion is related to the muscular tone. MYERS *et al.* (1913) state that creatinine excretion in different species of animals varies with the total amount of creatine in the muscles of the body. They regarded urinary creatinine simply as an index of muscle creatine.

Most surprising, however, were the results of BORSOOK *et al.* (1943). They found that phosphocreatine in the range of physiological H^+-concentrations yields, at 38°C spontaneously, *i.e.*, without the intervention of any enzyme, free creatinine at precisely the 2% observed by BLOCH *et al.* (1941) in the animal (rat) body. HOBERMAN *et al.* (1948), studying metabolism of creatine labeled with N^{15} in a human subject, came to similar results. They found that the turnover of endogenous creatine on a diet as free of creatine and creatinine as possible, is 1.64% per day.

BORSOOK *et al.* (1947) showed that most of the creatinine formed in the body arises from the mentioned spontaneous decomposition of creatine phosphate according to the equation: creatinephosphate $\rightarrow$ creatinine + inorganic phos-

phate, a reaction proceeding at a much faster rate than the formation of creatinine from free creatine under similar conditions.

Basing themselves on these results, BORSOOK *et al.* (1943) could easily explain the independence of creatinine from the amount of protein in the diet: The phosphocreatine content of muscle varies little; it then follows, since it is the immediate precursor of urine creatinine, that the latter will also vary little.

It is clear that this conclusion means an essential difference from the conception of FOLIN, who related the constant creatinine excretion as mentioned to the metabolic processes probably constituting an essential part of the activity which distinguishes living cells from dead ones.

Although FOLIN mentioned in the first place creatinine, he also considered neutral sulphur, and to a lesser extent uric acid and ethereal sulphate in urine, as representants of constant catabolism. However, it will be clear that, at present, the ethereal sulphates can no longer be considered as products of essential vital processes, because they are mainly detoxication products of aromatic substances originating from putrification processes in the intestinal tract. As regards the neutral sulphur in the urine, part of it is present in the amino acids cystine, cysteine and methionine, and, therefore, can neither be considered as a product resulting from vital activities. Moreover, it has been shown that neutral sulphur is not altogether independent of the diet. According to AMANN (1933) the neutral sulphur excretion is the same on "low" as on "normal" diets, but it is tripled when the dietary protein is increased 15 times.

On the other hand, the possibility cannot be rejected that the excretion of purine bodies, allantion and possibly other urine constituents is partly in accordance with FOLIN's conceptions. As a matter of fact, the names endogenic uric acid and exogenic uric acid have found general acceptance for many years.

It should, however, be stressed that the significance of the mentioned newer conceptions on creatinine formation should not be overestimated. Apart from any theoretical interpretation, it must be said that the constancy of creatinine excretion, independent from protein ingestion, is firmly established and must be taken into account in planning experiments on biological value of proteins.

The excretion of nitrogen on a protein-free diet amounts in a sheep to about 37 mg. per kg. per day; the excretion of creatinine-N can be estimated at about 8 mg., *i.e.*, 22 % of the total N. 78 % of the urinary nitrogen on protein-free diets is, therefore, bound in other compounds, mainly urea. It is evident that this urea originates from tissue protein, thus from endogenous catabolism. Nevertheless, variations in the daily excretion have been known for a long time. VOIT (1866) and other German investigators, in the latter part of the last century, had already discovered that the daily urea excretion of a fasting animal is possibly correlated with the amount of protein the animal had eaten before fasting. This dependence of urea excretion during fasting on preliminary protein feeding lasts, however, only a few days. This appears for example from three experiments conducted by VOIT with a dog. Before the three experiments the dog received 2500 gms., 1500 gms. and only a small quantity of lean meat respectively. During the experiments the animal was fasted and the urea excretion was determined with the following results (table 6):

The table shows that the urea excretions were very different in the first days of the experiments, but after some five days they were about the same.

From this and numerous other experiments VOIT and his contemporaries and immediate successors assumed that part of the protein in the body is very liable

ABLE 6. Urea excretion in a fasting dog on diets with different amounts of meat

Experiment	I	II	III
Meat ingested in preliminary period	2500 (gms.)	1500 (gms.)	little meat
Urea excretion in experiments (gms).			
1st day	60.1	26.5	13.8
2nd day	24.9	18.6	11.5
3rd day	19.1	15.7	10.2
4th day	17.3	14.9	12.2
5th day	12.3	14.8	12.1

to degradation and must be considered as a protein reserve. The ideas of that time were in 1905 summarized as follows by KELLNER in the first edition of his text book:

"Hieraus ist zu schliessen, dass das unmittelbar der Nahrung entstammende, in den Säften zirkulierende, gelöste Eiweiss viel leichter zersetzlich ist als das in den Organen abgelagerte organisierte Eiweiss. Dieser von C. VOIT zuerst nachgewiesene Unterschied zwischen *Zirkulationseiweiss* und *Organeiweiss* ist von der grössten Bedeutung für das Verständnis aller Vorgänge, welche mit dem Eiweissumsatz und mit der Fleischbildung im Zusammenhang stehen. Ohne uns darüber schlüssig zu machen, ob die erstere Form, das Zirkulationseiweiss, als "lebend" oder "tot", als Zellbestandteil oder nicht, aufzufassen ist, bezeichnen wir mit diesen Namen dasjenige Eiweiss, welches im ganzen Körper im flüssigen Zustande zirkuliert, alle Organe durchtränkt und auf seinem Wege unter dem Einflusse der Zellen einem raschen Zerfall unterliegt, während das organisierte Eiweiss der Gewebe eine weit grössere Beständigkeit besitzt. Bei Mangel an zirkulierendem Eiweiss, wie z.B. an den späteren Hungertagen, wird ein geringer Teil des Organeiweisses in Zirkulation gezogen".

The various German authors designated this labile protein by various names (unbekannte Mastsubstanz; zirkulierendes Eiweiss; Zirkulationseiweiss; Reserve-Eiweiss; Zelleinschlusseiweiss; totes Eiweiss; labiles Eiweiss; Vorratseiweiss). Some of these names have survived in the modern anglosaxon literature (circulating protein; protein reserve; protein store; reserve store).

In recent times the ideas on the "Zirkulationseiweiss" have revived and have been enlarged a great deal by the work of the groups of WHIPPLE, of ALLISON, of FROST and of others. The definitions have however been changed. As a rule the old investigators thought of storage of protein in the same way or approximately in the same way as that of carbohydrate and fat, either in the body fluids or extracellular and intracellular in the organs, especially in the liver.

At present, there can be little doubt that not only the "Zirkulationseiweiss" but also and even particularly part of the "Organeiweiss" of the German authors must be considered as belonging to the protein stores. The definition of MADDEN *et al.* (1940) for instance is as follows: "The reserve store of protein may be defined as all protein which may be given up by an organ or tissue under uniform conditions without interfering with organ or body function".

Extremely drastic means are applied to deplete the animals of protein and to measure the reserve store. MADDEN *et al.* mentioned that a normal dog is depleted of circulating plasma protein by daily plasmapheresis (bleeding and reinjection of the red blood-corpuscles suspended in a gum containing LOCKE solution) while consuming a constant basal diet low but adequate in protein.

With a normal dog it will be necessary to remove more plasma (and plasma proteins) in the initial days or weeks of the regime than in the subsequent weeks in order to attain and keep a steady hypoproteinemia. This excess quantity of plasma protein removed in the first weeks of a prolonged period of plasmapheresis represents the reserve store. The above mentioned authors estimate that normal dogs have sufficient materials in storage to form a quantity of plasma protein one or two times the amount which is normally present in their circulation. This may amount to as much or more than the total protein content of the liver and indicates that a large proportion of it must be stored elsewhere.

The method of FRAZIER *et al.* (1947) is also very drastic. In their later studies they depleted adult male rats weighing initially 288–350 gms. of 15–25% of their body weight in a period of 5 weeks on a protein-free diet.

MADDEN and WHIPPLE (1940) suppose that this reserve store will be drawn upon if the body need for protein to build new cell protein, new plasma protein, or new haemoglobin is greater than the exogenous supply. Thus, part of the body protein would form a reserve against adversity in the sense that it can be circumspectly depleted without apparent injury to the body. These stores would exert a protective function when the body is subjected to the strain of chloroform or arsphenamine poisoning and other destructive agencies, and behave as a bulwark against infection, bearing the brunt of sudden increases in protein catabolism due to inflammation, hyperthyroidism and probably other stresses.

As a revival of the old ideas as summarized by KELLNER may also be considered the opinion of the WHIPPLE-group on the existence of an exchange of protein materials between plasma and storage depots without splitting up the protein into single amino acids. In the opinion of WHIPPLE's group the blood plasma protein is part of a balanced system of body proteins: A "steady state" or "ebb and flow" or "dynamic equilibrium" exists between the plasma protein and a portion of the cell protein. This portion is the reserve store.

It has been shown also that in the animals deprived of their protein stores by the methods mentioned above there is a sensible loss of important enzymes in their livers, especially xanthine dehydrogenase, liver catalase, alkaline phosphatase, cathepsine and arginase. In our opinion such animals can no more be considered as normal.

All these facts are in agreement with the ideas of RUBNER (1911) and of DEUEL *et al.* (1928) on two types of deposit protein. One type is very labile and readily available. In humans for example who are subjected to a protein-free diet it is oxidized in some few days (BORNSTEIN *et al.*, 1928: 5–8 days; DEUEL *et al.*, 1928: 9 days). The other type is less labile and therefore less readily available, but more economically used. The subjects can only be deprived of it by drastic means such as plasmapheresis or by prolonged protein starvation. The first type corresponds with the Übergangseiweiss (transition protein) of RUBNER.

MARTIN *et al.* (1922) found that the daily excretion of the nitrogen from this transition protein with subjects on a protein-free diet can be represented by a simple logarithmic equation:

$$\log (y - \lambda) = a - kx,$$

in which x = the number of days,
y = the daily output of nitrogen in the urine,
λ = the minimum value of y,
a and k are constants.

If we consider two days, x_i and x_j, we have

$$\log (y_i - \lambda) = a - kx_i,$$

and

$$\log (y_j - \lambda) = a - kx_j,$$

so that

$$\log (y_j - \lambda) - \log (y_i - \lambda) = -k (x_j - x_i),$$

or

$$\log \frac{y_j - \lambda}{y_i - \lambda} = -k (x_j - x_i), \tag{1}$$

and

$$\frac{y_j - \lambda}{y_i - \lambda} = 10^{-k (x_j - x_i)}.$$

This means that the ratio $\frac{y_j - \lambda}{y_i - \lambda}$ is constant when $x_j - x_i$ is constant. Thus, every day, $y - \lambda$ is reduced to a constant fraction of its value of the day before.

The half value period $t_{\frac{1}{2}}$ of $y - \lambda$, *i.e.*, the period in which $y - \lambda$ is reduced to half its magnitude follows easily from the formula (1):

$$t_{\frac{1}{2}} = -\frac{1}{k} \log \tfrac{1}{2} = \frac{1}{k} \log 2.$$

MARTIN *et al.* calculated the constants a and k from the data of six experiments (four experiments with humans and two with pigs). They found:

$$\left.\begin{aligned} \log (y - 2.13) &= 1.142 - 0.330\, x, \\ \log (y - 2.13) &= 1.196 - 0.366\, x, \\ \log (y - 2.00) &= 1.153 - 0.331\, x, \\ \log (y - 2.00) &= 1.198 - 0.366\, x, \end{aligned}\right\} \text{humans}$$

$$\left.\begin{aligned} \log (y - 1.04) &= 1.080 - 0.180\, x, \\ \log (y - 2.66) &= 1.210 - 0.883\, x. \end{aligned}\right\} \text{pigs}$$

From these formulae we calculated

$$t_{\frac{1}{2}} = 0.91, 0.82, 0.91, 0.82, 1.67, 0.36 \text{ respectively.}$$

In every instance the half value period was therefore less than two days.

It is also easy to calculate the period $t_{1/100}$ in which $y - \lambda$ is reduced to 1/100. The formula is

$$t_{1/100} = -\frac{1}{k} \log \frac{1}{100} = \frac{1}{k} \log 100 = \frac{2}{k}.$$

We found

$$t_{1/100} = 6.1,\ 5.5,\ 6.0,\ 5.5,\ 11.2,\ 2.4 \text{ respectively.}$$

It may be concluded therefore that the highly labile transition protein is decomposed almost completely in 7 to 10 days.

It is however clear that the removal of the more stable protein stores takes much longer time. We already expressed our doubt whether the raiding of these relatively stable stores really occurs without interfering with organ and body function. Moreover, it seems highly probable that in this condition also the "Organeiweiss", that means the fixed body protein, will be more or less attacked.

We mentioned already that the WHIPPLE school holds that the protein store is not static, but that there exists an exchange or a dynamic equilibrium between the plasma protein and a portion of the cell and body protein. This exchange would take place without splitting up the protein into single amino acids.

The conception of exchange in this way is not essentially divergent from the older ideas. A perfect new theory, however, was established by BORSOOK *et al.* (1935) who felt that the theory of FOLIN (1905) was inadequate. Their experiments indicated that, over one day periods, whether the body nitrogen is spared by a protein or by nonsulphur – containing amino acids or ammonia, the same amounts of sulphur, and, by inference, of nitrogen are contributed to the urine by tissue sources.

From these and other experiments BORSOOK *et al.* in 1935, proposed a new theory of protein metabolism. They maintained that in an animal in nitrogen equilibrium the breakdown of intracellular protein is continually in progress, even when abundant quantities of amino acids are obtained from the diet. This breakdown bears no "wear and tear" connotation; it greatly exceeds FOLIN's endogenous quota and is directly proportional to the level at which the nitrogen balance has been set by previous dietary history. As a consequence, in nitrogen balance, a corresponding quantity of amino acids is synthesized into tissue proteins and peptides.

Thus, after this theory, breakdown and synthesis of tissue proteins are also proceeding in case of nitrogen equilibrium. BORSOOK *et al.* have termed this *continuing metabolism*, which means that the tissue proteins are in a dynamic state, irrespective of whether there is a nitrogen equilibrium or not. Even in the starved animal active resynthesis occurs.

According to the view of BORSOOK *et al.* the data indicate that the anabolic processes, continually in operation, must be more extensive than postulated formerly and in man normally would amount to 50% or more of the nitrogen intake. Although not stated explicitly, BORSOOK *et al.* seem to be of the opinion that this simultaneous protein synthesis and degradation refer only to part of the protein in the body, namely to the labile protein or reserve protein.

Still more revolutionary were the developments of SCHOENHEIMER *et al.* already mentioned before. Their findings with isotopes suggested that the biological system represents one great cycle of closely linked chemical reactions. This view of the living cell as a *dynamic system* was elaborated by these authors in 1939. They also pointed out that the living organism keeps constant the form of the cells and organs as well as the structure of the large molecules so that many investigators believe that the tissue enzymes which show their destructive power during autolysis lie dormant during life and are "activated" only when their function is required. The experiments with isotopes, however, indicate that all reactions for which specific enzymes and substrates exist in the animal are carried out continuously.

On several occasions MITCHELL (1943) and his group have tried to reconcile the conception of the dynamic state with FOLIN's theory of the independence of the endogenous and exogenous types of protein metabolism. They thought it likely that the chemical reactions between dietary amino acids and tissue proteins detected by SCHOENHEIMER do not relate to the fixed proteins of the cells, indispensable to their normal functioning, but to the dispensable reserve

proteins, readily subject to mobilization by many experimental procedures and as readily reformed. In MITCHELL's opinion, there is nothing in SCHOENHEIMER's work that denies the existence of a constant type of catabolism of nitrogen-containing compounds in the tissues. The beautiful analysis of the creatine-creatinine reaction performed by SCHOENHEIMER illustrates this very thing. And further: The term endogenous catabolism is still applicable, since its independence of the protein intake implies its restriction to fundamental constituents. Also, there is still a variable catabolism of nitrogenous substances, the rate of which is determined by the magnitude of the supply of dietary amino acids, regardless of whether this catabolism involves tissue constituents or dietary amino acids, and regardless of the proportions in which tissue and dietary constituents are involved. While the term in its original meaning is not descriptive of this phase of nitrogen catabolism, it may still be applicable in as far as the speed of the catabolism is set by the magnitude of the exogenous supply of nitrogen (MITCHELL, 1943). No well-demonstrated results in the field of protein metabolism have been found contradictory to FOLIN's concept; on the contrary they are best explained on this basis (MITCHELL, 1955).

In one of his latest publications, MITCHELL (1959) states that the reversible reactions revealed by isotope studies between tissue proteins and dietary amino acids are not anarchistic in nature. They seem to represent automatic and non-interruptable biochemical processes of synthesis as well as of degradation, which are balanced by an unknown regulatory mechanism so that the total amount of body material and its composition do not change (MOSS *et al.*, 1940). MITCHELL concludes that for the purpose of determining the amino acid requirement the tissue proteins may be considered to be static.

Indeed, in continuing isotope studies, it turned out that the rates of protein rejuvenation are quite different in various species and various organs. SPRINSON *et al.* (1949) found that SCHOENHEIMER's original observations of the extreme instability of cellular proteins are more typical for the rat (half value period = 17 days) than for the human (half value period = 80 days). While the proteins of the internal organs, both in the rat and in the human, are extensively involved in the dynamic state, this appears to be far less so in the muscle proteins. According to RITTENBERG (1948/49) the great mass of muscle protein must be metabolically quite inert, being a mere frame-work to which certain active proteins are attached. He considers it possible that within each cell structures exist which, like the red cell, are metabolically inert. Among them may be those in the brains whose function is to store memories of the past.

RITTENBERG concludes: once again we see an example of the turning wheel. Before the isotope technique was developed, the larger part of the cell was considered inert. This theory is described best by its name, the theory of wear and tear. The initial experiments with the isotope technique which, because of the expense of isotopic materials, were carried out with rats, resulted in the promulgation of the theory of the dynamic state. We now are swinging back to a point somewhere in between. The organism is partly in the dynamic state and partly in an unreactive one. The relative division depends on the particular species and the particular organ studied. It would be unwise to consider the theory of the dynamic state as having been overthrown. Rather it has been amplified and developed.

ALBANESE (1959), to a certain extent, joins the ideas of MITCHELL. Admitting the possibility that the distinction between exogenous and endogenous nitrogen

as envisionised by FOLIN (1905) is no longer tenable according to the dynamic concept of protein metabolism, he adheres nevertheless to the opinion that, in no way, the results secured by the THOMAS-MITCHELL method of determining the biological value are invalidated.

Although it seems that the divergency of opinions on protein metabolism has disappeared to a great deal, there remain several open questions. First of all there is some confusion about the question how the endogenous nitrogen excretion should be defined. Some authors consider as endogenous all degradation products originating from body proteins, reserve stores included. BURROUGHS *et al.* (1940), however, claim that in any study on endogenous metabolism the stores of nitrogenous substances, whether protein or not, must be reduced to zero by continued feeding of diets containing only inconsiderable amounts of nitrogen. This deposit protein is also the cause that the urinary nitrogen does not immediately adjust to endogenous levels in subjects removed from normal rations to rations containing little or no nitrogen (MITCHELL and HAMILTON, 1929).

The question is, however, more complicated than was supposed formerly, because, as mentioned, there proved to be two types of protein stores, one very labile, being removed in 7 to 10 days on a protein-poor diet, and a second one which is delivered up very reluctantly. It is therefore probable that in most experiments on the determination of biological values the true endogenous excretion has not been attained during the protein-poor control periods. This seems, however, not to be too serious because the abrading of these less labile protein stores takes place so slowly that the daily nitrogen excretion originating from this type of nitrogen is only small.

Another more theoretical question is as follows. According to a theory developed by RITTENBERG, the dynamic state relates mainly to the protein stores and not to the inert frame-work of the cells. It is clear that this may be verified by isotope experiments on thoroughly depleted animals. As far as we know such experiments have not yet been performed.

Finally, we come to the question whether the endogenous nitrogen excretion as defined by MITCHELL is really constant. This question has often been examined and, unfortunately, it has been shown that there may be circumstances in which the constancy does not hold. Even MITCHELL *et al.* (1929) distinguish two types of endogenous catabolism: the "minimum" endogenous catabolism, and a second type termed the "accelerated" endogenous catabolism. Fortunately, the latter is a sporadic phenomenon resulting from caloric undernutrition, exophthalmic goiter, diabetes, or poisoning by certain poisons. MUKHERJEE *et al.* (1949) also mention hyperthyroid activity as cause of accelerated endogenous metabolism. In 1955 MITCHELL added impairment of liver function and raiding of tissue to supply feather and hair growth. These conditions (MITCHELL *et al.*, 1929) involve the destruction of tissue proteins. The end products resemble those of the exogenous catabolism in consisting largely of urea and in containing no creatinine.

Most of these conditions are pathological. It should, however, be kept in mind that an animal depleted from all its labile and less labile protein stores and with impaired enzyme systems cannot be considered as being normal and in good health. This is also a reason for not going too far in robbing the animals

completely from their protein stores during experiments on the determination of biological value.

A number of attempts to measure the minimum endogenous catabolism have been reported in the literature; many of them have been carried out with human subjects. There is a good deal of variation, from 0.0594 gm. down to 0.0241 gm. of N daily per kilogram of body weight, among these determinations with men (DEUEL *et al.*, 1928). The values for pigs (0.035 to 0.072, MCCOLLUM *et al.*, 1913) bear a remarkable resemblance to those for humans. The values for cattle (0.029–0.045) and for sheep (0.024–0.072) (MITCHELL *et al.*, 1929) are of less significance than those for pigs, because of the difficulty of inducing these animals to consume N-free rations in adequate amounts for any considerable period of time. Nevertheless, they are of the same magnitude as the values for pigs.

The minimum endogenous urinary N of dogs (0.092–0.173 gms.) and of rabbits (0.105–0.141) per kg. of body weight seems to be considerably higher than those for other animals. The same is true for the rat (0.110–0.219) as reported by MITCHELL *et al.* (1929). The variations existing among the determinations are apparently due to individuality and to approximate success in most of the experiments in reaching the actual endogenous level of excretion.

Apart from the nutrition history and heriditary factors of the organism it has been found that while the endogenous nitrogen (*EN*) per unit of body weight definitely tends to decrease with increasing weight and age (MITCHELL *et al.*, 1929), the *EN* is constant when related to basal metabolism. This correlation seems to prevail not only for men, but also for pigs, cattle, sheep, dogs, and rats and may be due in part to hormonal or enzymic factors (ALBANESE, 1959).

As we have already mentioned, it is very difficult to conduct such experiments with rations devoid of N with ruminants. Nevertheless some authors have succeeded in feeding sheep on a nearly N-free diet. They refer the endogenous urinary nitrogen to kilogram body weight. Their results are shown in table 7. The values of HAMILTON *et al.* (0.0276) and of HUTCHINSON *et al.* (0.0630) which show large deviations from those reported by the other authors are nevertheless included.

TABLE 7. The endogenous urinary nitrogen on N-free diets with sheep as found in the literature

Name of Author	Year	Kind of animal	Endogenous nitrogen (gm. per kg. of body weight)
RITZMAN *et al.*	1930	sheep	0.0333
SOTOLA	1930	lambs	0.0331
HUTCHINSON *et al.*	1936	sheep	0.0630
SMUTS *et al.*	1938	„	0.0350
MILLER *et al.*	1939	lambs	0.0370
HARRIS *et al.*	1941	„	0.0333
HAMILTON *et al.*	1948	„	0.0276
Average	–	–	0.0372 (corresponding to 0.231 gm. Protein)

In calculating the *BV* with the THOMAS-MITCHELL formula a figure for *EN* must be used. For these calculations we made use of the results obtained by various authors in experiments with sheep on N-free or nearly N-free rations. The average of 0.037 gm. per kg. of body weight thus obtained will be considered as the most reasonable one for computing the *BV* of the protein in our experiments.

CHAPTER V

METHODS FOR DETERMINING THE BIOLOGICAL VALUE OF PROTEINS

In feeding experiments with dogs MAGENDIE (1841) showed that there are differences in the nutritive value of proteins. He found in trials that dogs could not be maintained or failed in health when given gelatin or bread and gelatin as contrasted with meat (MCCOLLUM, 1956).

In the Netherlands, G. J. MULDER (1847) did not consider animal and plant protein as being fully equivalent. This he expressed in the following manner: "Meat furnishes a stimulus – to use a general expression – that cannot be substituted by any plant protein compound" (BROUWER, 1952). Many years later, C. VOIT (1872) came to a similar conclusion as MAGENDIE regarding gelatin.

The term biological value of a protein was introduced by THOMAS (1909) and the idea was extended by MITCHELL (1924) to describe the quality of protein. It may be defined as the percentage of absorbed N that the body saves from being excreted in urine and faeces. Most of the data for biological value have been obtained with rats. Several other studies have been made with pigs and chickens and a few with sheep and cattle.

The biological value of protein in feeds in the case of ruminants involves to some extent the synthetic activities of the flora and fauna of the paunch. The rumen microorganisms possess a capacity of synthesizing their own cellular proteins from the feed proteins and even from the non-protein nitrogenous constituents of the paunch contents as has been explained in chapter II. These proteins are carried through the digestive tract of the animal and digested and utilized in the same manner as are feed proteins. Other feed proteins are broken down to ammonia and volatile fatty acids.

Several methods for determining the biological value of proteins are mentioned in the literature:

A. DESCRIPTION OF METHODS

1. *The nitrogen balance method*

In measuring the biological value (*BV*) for growth, the percentage retention of the absorbed N (food N minus urine and faecal N) has been calculated by the formula:

$$BV = 100\,\frac{I-(UN+FN)}{I-FN},$$

where I = N intake; UN = N in urine and FN = N in faeces.

The numerator represents the N-balance and the denominator the N digested. MITCHELL (1924), however, pointed out that this method fails to furnish an absolute measure of the *BV*, since it neglects one purpose for which protein is constantly being used in the body, namely the maintenance of the tissues. The shortcoming of the formula is that N used in maintenance (endogenous N) is not included in the numerator, whilst, in the denominator the *requirement* of N for endogenous N is actually comprised.

2. *The Thomas-Mitchell method*

A more exact and less variable measure of the nutritive value of a dietary protein is formulated by THOMAS (1909). He determined in experiments on himself his output of urine-N on a protein-free diet, and on several test diets. He established three formulae for the computation of biological values involving three different assumptions concerning the amount of metabolic N in the faeces.

In his first formula he assumed that none of the N in the faeces was metabolic. If *BV* stands for biological value, *UN* for N in urine, *EN* for endogenous N (which is N in urine on a N-free diet), *FN* for faecal N, *MFN* for metabolic faecal N, *B* for N balance end *I* for N intake, this first formula was:

$$BV = 100\,\frac{EN + B}{I - FN}.$$

In the second formula he assumed all the N in the faeces to be metabolic:

$$BV = 100\,\frac{EN + FN + B}{I}.$$

These two formulae do not yield good results with food containing much cellulose. Therefore, he composed a third formula in which he assumed the metabolic faecal N to be 1.0, as in the case of vegetables:

$$BV = 100\,\frac{EN + B + 1.0}{I - FN + 1.0}.$$

MITCHELL (1924) extended the third formula by introducing a better correction for metabolic faecal N, thus:

$$BV = 100\,\frac{EN + B + MFN}{I - FN + MFN}.$$

Considering that *B* stands for $I - (FN + UN)$, MITCHELL, after substitution and rearrangement, writes:

$$BV = 100\,\frac{I - (FN - MFN) - (UN - EN)}{I - (FN - MFN)}.$$

BROUWER (1959) proposed still another rearrangement of Mitchell's formula:

$$BV = 100 - 100\,\frac{UN - EN}{I - FN + MFN} = 100 - 100\,\frac{UN - EN}{\text{absorbed N}}.$$

From this formula it is clearly seen that the biological value is simply the percentage of absorbed N that escapes from being excreted in the urine.

The values for *MFN* and *EN* cannot be determined while the protein in question is under investigation, but they must be calculated from values obtained in separate periods when the animals are receiving an N-free diet as previously explained in chapters III and IV.

Some investigators used the N-free diets at the initiation of experiments (MITCHELL, 1924 and 1928). MITCHELL however pointed out that the determination of the *BV* is unaffected by the position of the experimental period in relation to the near-by nitrogen-free periods. Others proposed to employ a nearly N-free ration both before and at the conclusion of the experimental series of rations (SOTOLA, 1930 and 1933; TURK *et al.*, 1934; SMUTS *et al.*, 1938

and LOFGREEN *et al.*, 1947), whilst MILLER *et al.* (1942) suggested that the period of the low-N feeding should be conducted at the end of the experiment and not preceding or during the course of the test periods. They also advised that the animals having gone through a low-N ration should be discarded from future experimental use.

In order to avoid the difficulties experienced with animals to eat low-N diets, some workers, in computing the biological values, have used average values reported in the literature for *MFN* losses (per unit of dry matter consumed) and *EN* losses (per unit of body size). It was however found that it makes little difference whether the biological values are calculated with the average values or with the actually determined ones (LOFGREEN *et al.*, 1947).

The THOMAS-MITCHELL method is the most wide-spread procedure for measuring the *BV* of proteins. SOTOLA (1930) concluded that it is the most accurate method for evaluating proteins. RIPPON (1959) is of the same opinion.

3. *The protein efficiency ratio*

The THOMAS-MITCHELL method requires exact metabolism data. For the determination of the protein efficiency ratio as developed by OSBORNE *et al.* (1919) however, it is only necessary to weigh the feed consumed and the animals (mostly young growing rats) during the course of the experiments. From these data the protein efficiency ratio *R* or "growth promoting value" can be calculated with the following formula:

$$R = \frac{\text{gain in weight}}{\text{intake of protein}}.$$

Although in the early days of modern protein research many striking results have been obtained with this method, it has serious drawbacks.

One of them is the same as mentioned above in our discussion on the nitrogen balance method: The neglect of the endogenous nitrogen. Again in the numerator *EN* is not included, whilst in the denominator *the requirement* of N for *EN* is actually comprised. This is one of the reasons why the magnitude of the protein efficiency ratio is highly dependent on the protein level in the feed.

A second drawback is that increase of body weight is a poor measure for protein growth.

For both reasons absolute data cannot be obtained, only comparative ones, provided that the experiments are conducted under highly standardized conditions. OSBORNE *et al.* (1919) mentioned that the proteins should be compared by assessing the level in the diet at which the maximum value for *R* is obtained. The relative value of the proteins compared would then be inversely proportional to these levels. Others prefer all test rations to have exactly the same protein level.

Further drawbacks are that usually the digestibility of the proteins is not taken into account and that large numbers of animals are required.

Several modifications and refinements of this method have been proposed and are also used until now with fairly good results (RIPPON, 1959). We refrain however from entering into details, because all these methods can hardly be used in experiments with ruminants.

4. *The protein minima*

The method of the protein minima was used by MELNICK *et al.* (1936, 1937) in

their experiments with adult dogs on synthetic diets. They determined the minimal amounts of protein in the diet necessary to maintain equilibrium, in order to obtain figures for the maintenance value of these proteins. They were however aware of the limitations of this method, mentioning that the minimum value depends on many factors, such as:

a) The nature of the foodstuffs fed with the protein,
b) The completeness of the diet, both qualitatively and quantitatively,
c) The caloric value of the food given,
d) The degree of maturity of the experimental animal,
e) The activity of the experimental animal,
f) The environmental temperature,
g) The nutritive condition of the animal, and
h) An adequate preliminary adjustment period.

Therefore they considered it necessary to begin with a low protein diet entailing a distinctly negative balance. After that, by repeated protein increments at the expense of an isodynamic portion of the carbohydrate fraction, they attempted to attain equilibrium. Such a procedure was considered essential since N-equilibrium may occur at different levels in a normal animal, provided the protein intake is above the amount minimal for N-equilibrium (VOIT, 1881). The experimental periods usually lasted only 4 days, the preliminary and intermediate adjustment periods about 7 days. One experiment with 3 or more experimental periods, therefore, took one to two months.

Although not giving proper figures for biological values in the sense of THOMAS-MITCHELL, the method nevertheless seems promising for studies with ruminants. The experiments will however be time-consuming, because in investigations with ruminants, the periods must be much longer than in experiments with dogs. Moreover, it should be borne in mind that the limitations enumerated above deserve close consideration.

5. *The egg replacement value*

This method can be considered to be based on the nitrogen balance method. Earlier experience led MURLIN and co-workers (1938a, b) to the conviction that a proper application to human subjects of the strict biological value method as worked out by MITCHELL (1924) for rats would always entail such difficulties as to jeopardize the results. Because of the unpalatability of the N-free diet and its tendency to induce diarrhea in human subjects, they decided to eliminate as far as possible the inequalities of the control and experimental diets by keeping the proportions of protein, fat and carbohydrate as constant as possible. Milk or egg were chosen as standards of high protein value and included in the control diets. In alternate periods a comparison was made between the N-balances on diets with milk or egg proteins and with the protein to be examined. Mostly egg protein was used for this comparison.

The egg replacement value (R_v) of a protein (P) is calculated by dividing the difference in N-balance on the two diets by the amount of egg-N fed. This difference, as a percentage, is then subtracted from 100 to find the extent (in percentage) to which (P) replaces egg. Thus:

$$R_v = 100 - 100\,\frac{B_1 - B_2}{I},$$

where B_1 and B_2 are the N-balances on the diets with egg protein and with the protein under investigation.

The replacement value has been used successfully for determining the nutritive value of protein in men and animals. Its value in experiments with ruminants is however questionable, because the nutritive value of milk protein and probably also that of egg protein is by no means as high as in experiments with non-ruminants (see chapter II). At present it is impossible to point out a more suitable reference protein.

6. The rat repletion method

Because the determination of growth rates in young rats and the estimation of nitrogen metabolism are time-consuming procedures, CANNON *et al.* (1944) developed an additional method which depends upon the principle of feeding known amounts of protein over a period of 7 to 14 days to adult-deficient male albino rats and ascertaining the effects upon weight recovery and regeneration of total serum protein. Gain of haemoglobin, carcass protein and liver protein have also been used as criteria. The method can demonstrate variations in protein quality in one or two weeks. This presupposes a supply of protein-deficient animals kept on a basal ration practically devoid of utilizable protein until 25% of the original weight is lost. To this basal ration the protein to be tested is added at a 9% protein concentration.

To us it seems that this method is hardly suitable for experiments with ruminants because the value of a protein for regeneration of serum protein and of haemoglobin may be different from that for regeneration of the body tissues. A better criterion would be carcass gain. However, for this many animals would have to be sacrificed. Moreover, the method yields only comparative values and even CANNON himself (1945) calls it only a sort of "screen test".

7. The nitrogen balance index

ALLISON *et al.* (1945, 1946) introduced this method for measuring the nutritive value of proteins. This index has been defined as the tangent of the curve relating N-balance to absorbed-N. This method was suggested to establish more clearly over a wide range of values the relation between absorbed food N, N-balance, and *BV* of proteins. ALLISON *et al.* found that the relationship between N-balance and absorbed-N in normal adult dogs was linear in the region of a negative N-balance, and that the linearity often extended over onto the positive side, but became curvilinear on the positive side of the N-balance. The equation describing the linear portion is:

$$B = k.\,AN - EN,$$

where B is N-balance, AN is absorbed N, EN is the excretion of N on a protein-free diet (the sum of urinary and faecal N) and k is the slope of the line. In the linear region k may be found from two experiments indicated by 1 and 2:

$$k = \frac{(B_2 - B_1) + (EN_2 - EN_1)}{AN_2 - AN_1}.$$

When B_1, EN_1 and AN_1 refer to a protein-free period, k is identical with the *BV* of MITCHELL. In general k may be considered as a measure of the *BV* obtained without having recoursed to protein-free periods. The condition however is that in experiments 1 and 2, dry matter intake and live weight are the

same so that *MFN* and *EN* also remains unchanged. Apart from this restriction the nitrogen balance index method may be understood as an extension of the THOMAS-MITCHELL method.

As in our experiments the diets were never completely free from protein, our method of determining *BV* has been something between the MITCHELL- and the ALLISON-method.

8. *The chemical score method*

Previous attempts to relate the amino acid content of food proteins to their *BV* have focused attention on the limiting essential amino acids. For example, MITCHELL *et al.* (1946) compared the contents of the essential amino acids in single proteins and in the mixed proteins of foods with those of egg protein, by computing the percentage deviations for each amino acid. The nutritive value was expressed as a "chemical score", equal to the highest percentage deficit of an essential amino acid in the protein or protein mixture under scrutiny. This score equals 100 for any protein or protein mixture completely lacking in any one essential amino acid or it equals zero if the score is taken as 100 minus the highest percentage deficit as later was proposed by the same authors.

It is well known that the determination of the amino acid composition is easier and quicker than the biological determination in animal tests. Nevertheless, the variability of the results of chemical score determinations was found to be relatively large. Moreover, the score depends on only one single amino acid. This is indeed a drawback because the *BV* of a protein is not exclusively dependent on the amino acid with the largest deficit, but to some extent also on the other amino acids. It has namely been shown that also the mutual ratios of the amino acids may affect the *BV* of a protein.

9. *The method of the "Essential Amino Acid Index"*

More attractive seems the method of OSER (1951, 1959), who proposed to score the nutritive value of proteins by the computation of the "Essential Amino Acid Index" (*EAAI*), in which all the essential amino acids are involved. OSER considered that the probability of two or more events occurring simultaneously is a function of the product of the probability of their individual occurrences. It follows therefore that the probability that all the amino acids will be available at the site of synthesis (that is to say for biological utilization) is a function of their product.

OSER adopted egg protein as a standard and calculated the percentage ratios of the amino acids in food protein relative to their respective contents in whole egg proteins: "egg ratios". The Essential Amino Acid Index (*EAAI*) was defined as the geometric mean of these egg ratios:

$$EAAI = \sqrt[n]{\frac{a_p}{a_e} \times \frac{b_p}{b_e} \times \ldots \times \frac{n_p}{n_e}},$$

where a_p, b_p, ..., n_p are the percentages of each of the ten essential amino acids in the food proteins and a_e etc., are those in the egg protein.

EAAI is readily computed logarithmically as follows:

$$\log EAAI = \frac{1}{n}\left\{\log \frac{a_p}{a_e} + \log \frac{b_p}{b_e} + \ldots + \log \frac{n_p}{n_e}\right\}.$$

For the calculations with these formulae two more assumptions were adopted, namely that the minimum ratio of an amino acid content relative to that of the standard protein is 1% and the maximum is 100%.

Regression equations were derived in order to calculate the *BV* from *EAAI*. There proved to be a general agreement between calculated and observed estimates even though the samples upon which these estimates were based were different (OSER, 1959). With the aid of these equations the *BV* may therefore be predicted from the amino acid pattern although there are some limitations.

Especially in our case one of these limitations is valid: It is extremely difficult to take into account the digestibility of the protein in roughages. Moreover, OSER's formula only predicts the *BV* for monogastric animals. For ruminants it cannot be used as the *BV* may seriously be modified by the bacterial processes in the rumen.

10. *The method of "Net Protein Utilization"*

MILLER *et al.* (1955) have devised a method which is called the method of "Net Protein Utilization" (*NPU*).

Groups of rats weighing 50–60 gms. each are fed on an equicaloric non-protein diet and on test-protein diets for 7 to 10 days. At the end of the periods the animals are killed and the water contents of the bodies are determined. Then the N contents are calculated from the water contents with the aid of regression formulae. Nitrogen estimations are considered unnecessary. After that, the *NPU* is calculated by applying the equation of BENDER *et al.* (1953):

$$NPU = \frac{B - (Bk - Ik)}{I},$$

where *B* and *Bk* are the total body N of the test and "non-protein" groups respectively, and *I* and *Ik* are the intakes of N in the two groups. (*Ik* is presumably only a small correction).

MILLER *et al.* (1955) also computed a figure for *BV*, for which the protein digestibility (*D*) has to be taken into account:

$$D = \frac{I - (F - Fk)}{I},$$

where *F* and *Fk* are the faecal N values on the test and non-protein diets respectively, and *I* is the intake of N on the test diet. It is clear that *Fk* mainly represents metabolic faecal nitrogen, so the numerator equals:

$$\text{N intake} - (\text{faecal N} - MFN)$$

in MITCHELL's formula on the biological value, and *D* must be considered as the true digestibility. The *BV* follows:

$$BV = \frac{NPU}{D}.$$

The advantage of the method described here is the small number of measurements to be made and the brevity of the experimental periods. The method is however unsuitable for testing net protein utilization and biological value of proteins in ruminant nutrition, because all experimental animals have to be sacrificed.

11. *Other methods*

MURLIN *et al.* reported in 1953 on correlations arrived at between biological values of proteins and creatinine N percentages in the urine. In their experiments with dogs these correlations led them to the prediction of biological values from creatinine N percentages.

Another method for estimating the *BV* of proteins was reported in 1957 by DJU *et al.* when observing good correlations between the *BV* and the activity of liver enzymes, particularly xanthine oxidase. They applied this method in their experiments with rats especially in cases of diets normally producing diarrhea in the rat. They mentioned that previous protein depletion of the adult rat did not increase the sensitivity of liver xanthine oxidase activity as an index of protein quality.

These two methods have not yet found general acceptance but should be tested thoroughly.

Conclusion: Although there are numerous methods for determining the biological value, it is clear that, in our case, those methods in which large numbers of animals have to be sacrificed were out of the question. Neither was the method of the egg replacement value suitable, because the reference substance might be degraded by the rumen bacteria. We adhered therefore to the balance sheet methods, especially that of THOMAS-MITCHELL in various modifications, and also to the nitrogen balance index. The method of the protein minima might also have been useful, although there are some inconveniences as already has been pointed out.

B. FACTORS AFFECTING THE *BV* OF PROTEINS

Among the important factors that affect protein utilization and thus its *BV* are: the physiological processes for which proteins are necessary, the level of protein fed, the species of animals, the digestibility and the energy intake.

1. *The physiological processes*

The concept of biological value (*BV*) was originally applied by THOMAS (1909) to the protein requirements for maintenance of the adult individual. MITCHELL (1924) has extended this application to growing animals requiring protein for growth as well as for maintenance.

As protein consumption increases, the fraction of absorbed protein utilized for maintenance decreases, whilst the fraction utilized for growth rises to a maximum and then falls because more and more protein is only used for the production of heat. Thus, there is a decrease in *BV* as the protein consumption increases.

Yet, for the *BV* for maintenance alone, fairly accurate figures can be obtained. This can be done by determining the amount of protein from different sources required to maintain the nitrogen equilibrium. Of course, the rations must be adequate in all other respects. With this intention many experiments have been performed with rations at a 5% protein level, because it seems that this level is close to the actual maintenance level. HEGSTED *et al.* (1946) suggested that from 3 to 4% of protein suffices for maintenance; from 15 to 20% is usually required for optimum growth.

MITCHELL recognized as far back as 1924 the obstacle in the path of any method of determining a separate *BV* for physiological processes such as growth, reproduction and lactation necessarily superimposed upon the function of maintenance. The difficulty would be largely due to the qualitative differences between amino acid requirements for maintenance and for these functions.

BARNES *et al.* (1946) drew the conclusion that the *BV*'s for growth and maintenance should be measured independently and not in combination. They did not however mention how the *BV* for growth can be measured separately. As a matter of fact, exact figures for the *BV* of growth alone, cannot be obtained; they always refer to growth plus maintenance. Neither have methods been devised to correct for maintenance. For the same reason MITCHELL (1924) concluded that it is impossible by any but indirect and approximate methods to obtain a *BV* of food proteins for the process of milk production. In such cases only biological values for maintenance plus growth, and for maintenance plus milk production can be obtained. MORRIS *et al.* (1936) have devised a formula for correcting for milk production. However, several assumptions had to be made so that it does not seem suitable to enter into details.

Although, for all these reasons, there are no exact figures for the *BV* for growth alone, MITCHELL (1924) considers it as obvious that lysine and cystine are required in relatively larger amounts for growth than for maintenance. For growth, casein has been shown to be superior to edestin while for maintenance the reverse is true (OSBORNE *et al.*, 1916). Biological values of 76 and 40 were secured by MITCHELL *et al.* (1950) for beef muscle and wheat gluten proteins respectively for growth plus maintenance, but for maintenance alone the values were nearly equal, being 69 and 65.

2. *The level of protein fed*

Figures for biological values show indeed that the results depend upon the concentration of protein in the diet: The ratios of the *BV*'s of different proteins depend on the protein levels of the rations. For instance, MITCHELL *et al.* (1923) mentioned that "while it is probably true that all proteins have lower biological values for growth than for maintenance, our experience has shown that the level at which the protein is fed is probably of greater importance in most cases in determining its value to the body in satisfying nitrogen requirements than the purpose for which it is used in the body". He added that in covering the maintenance requirement the *BV* of a protein seems to be lower when fed at a 10 percent level than when fed at a 5 percent level, so that at the former level the difference in the values of the protein for maintenance and growth is probably not great. This however is not so strange because with 5% or even less of most proteins the maintenance level has already been attained, as mentioned before (HEGSTED *et al.*, 1946). MITCHELL *et al.* (1923) reported also that while their experience with rations containing more than 10 percent of proteins was limited, such as it was, it indicated that no marked further decrease in *BV* need be expected until the level is so high that maximum growth is possible. PLATT *et al.* (1958) showed also that the *BV* of protein is not static and may alter with changes in the concentration of protein in the diet as may be seen from the following table (8).

From experiments with growing rats which were fed rations with various protein levels (4, 8, 12, 16, 20, 24 and 28%) FORBES *et al.* (1958) concluded that in general the relationship between *BV* and protein concentration was linear.

TABLE 8. Variation in biological value of egg protein and casein when given at different levels to weanling rats

Percentage of protein in the diet		4	8	10	12	16	20	30	40	56
Biological value	Whole egg	100	91	–	84	62	53	–	42	20
	Casein	–	60	60	58	–	46	38	–	–

The equations expressing this relationship are:

$$y = 126.5 - 2.73x \quad \text{for whole egg,}$$
$$y = 96.1 - 1.78x \quad \text{for casein, and}$$
$$y = 75.3 - 1.35x \quad \text{for peanut meal,}$$

where y is the BV and x is the percentage of protein in the diet.

With levels below maintenance the BV seems to be constant as mentioned before.

3. *BV for different species of animals*

Most authors seem to favour the view that different species (rat, pig and chicken) utilize protein in metabolism with about equal efficiency (HART *et al.*, 1919).

SMUTS *et al.* (1939) reported that digestibilities as well as biological values of white fish meal are lower in sheep than in rats. However the difference in BV might be due to the different protein levels. The protein percentage in the feed of the sheep was approximately 14%, while it was only 9% in the ration of the rats. However, the authors also consider it possible that the fermentations in the alimentary canal have contributed to this difference. Many authors (JOHNSON *et al.*, 1942, 1944; MCDONALD, 1952; OYAERT, 1954 and ELLIS *et al.*, 1956) maintained that the amino acid pattern and the BV as determined with rats has little significance for the ruminant because the feed protein would be converted nearly completely to microbial protein. In chapter II it has however been explained that with some proteins the conversion is far from complete.

4. *The digestibility*

From the work of BROUWER, DIJKSTRA, HUISMAN, AXELSSON and many others (chapter III) it is well known that lower protein contents in the feed are associated with lower apparent protein digestibility and conversely. Thus protein content is of primary importance in the determination of the BV with the aid of the method of the protein efficiency ratio. In this method digested and absorbed protein are generally not taken into account, only the protein level in the feed.

It is however very improbable that the protein level and the digestibility in this way are affecting markedly the figures of BV as determined by the method of THOMAS-MITCHELL. Their formula does not make use of the protein level but of the amount of absorbed protein. Changes in protein level and apparent digestibility are therefore duely allowed for.

5. *The energy intake*

Maximum utilization of proteins can only take place when the energy needs are satisfied by fat and carbohydrate. Moreover, the essential amino acids should be simultaneously available in correct proportions, together with ade-

quate amounts of other constituents such as vitamins and minerals. Several workers have demonstrated that N retention was improved by increasing carbohydrate and fat intake. MITCHELL (1943), FORBES *et al.* (1955) and FORBES *et al.* (1958) reported however that the effect of energy intake on *BV* seems only important in the extreme situation when proteins must be called upon to provide energy for vital functions.

It is clear that all these factors must be considered when using methods for determining the *BV* of grass hay with sheep. Accordingly, the protein level of the feed was taken so moderate that the absorbed protein was mainly used for maintenance and only to a small extent for growth. The energy intake was high enough to warrant that there was no need for the animals to call upon proteins in order to provide energy for vital functions. Moreover, the influence of the relation between protein level and digestibility on *BV* was duely eliminated by the choice of the balance sheet method of THOMAS-MITCHELL and related procedures.

CHAPTER VI

BIOLOGICAL VALUE OF PROTEINS IN ROUGHAGES

It is well known that pasture and hay are different in various countries. This refers to the botanical as well as to the chemical composition. Both are different in dry and wet climates, in warm and in cold regions, etc. It seems therefore questionable whether observations in foreign countries can learn us very much about the biological value of hay protein in the Netherlands. Nevertheless, we considered it useful to give a review of the biological value of herbage protein in other countries, the more so as such determinations have not been carried out until now in the Netherlands.

Few experiments have been conducted on the biological value of the protein in common roughages, although most ruminants subsist only on pasture. Even when pasture is not available, roughages such as hay and silage and their proteins are essential parts of most rations for ruminants.

It is true that lush pasture is rich in protein and provides the animals with a surplus of protein so that the biological value of the nitrogenous substances is of minor importance. In the last stages of growth, however, the protein of pasture may be very low. This is the reason why in dry countries the protein intake of the animals may be very low. In such cases the biological value of pasture protein might be of considerable importance. The same refers to the hay in seasons when pasture is not available and the animals subsist on hay only or on hay, silage, roots and concentrates. More data on the biological value of hay protein is therefore highly desirable.

At one time the biological value of protein was considered to be equal in all feeds for ruminants. From their own investigations and from a literature review JOHNSON *et al.* (1942, 1944) suggested that all crude protein fed at a level of 10 to 12% would for ruminants have biological values approximating 60. We have mentioned already in chapter V their suggestion that this would be due to the conversion of feed protein to microbial protein. They assumed that, for sheep, bacterial protein has a biological value of 60.

Contrary to the findings of JOHNSON *et al.*, several other workers have re-

ported biological values greater than 60 and also significant differences in the biological values of various nitrogenous supplements when fed to sheep. For example, LOFGREEN *et al.* (1947) have found that this generalization is not correct. They supplemented a timothy hay and maize ration with urea, urea plus methionine, linseed oil meal, and whole egg protein. These diets contained 10% crude protein on air-dry basis, 40% of which consisted of the various supplements. Biological values of 71, 74, 76 and 80 were obtained for these rations respectively.

WILLIAMS *et al.* (1951) have confirmed and extended the work of LOFGREEN *et al.* by experiments with sheep on a basis ration of timothy hay and maize. Supplements of urea, urea plus methionine, linseed oil meal, subterranean clover seed, casein, and whole egg protein supplied 40% of the total N. The crude protein content of all the rations was very close to 10%. The biological values obtained were 68.7, 75.2, 79.7, 83.0, 82.0 and 86.7 respectively.

We now consider the results obtained with ruminants on diets containing only protein from roughages. Most of these results refer to lucerne hay fed to sheep on maintenance rations. Very few have been performed with grass products such as grass hay.

SOTOLA (1930) has studied the *BV* of proteins in lucerne hay, sunflower silage, and maize silage when fed to lambs as a single feed and in combination. The order of feeding was as follows: (1) nearly N-free rations, (2) lucerne hay, (3) sunflower silage, (4) 1 part of lucerne hay, 3 parts of sunflower silage, by weight, (5) maize silage, (6) 1 part of lucerne hay, 3 parts of maize silage, by weight, and (7) nearly N-free rations. The nearly N-free rations consisted of straw, starch, sugar and oil. The average dry matter intake per lamb during the low-N periods was only 273.8 gms. daily.

If in the calculations allowance was made for the results of the low-N rations, figures of 56, 67 and 94 were secured for the *BV* of lucerne hay, sunflower silage and maize silage, with protein levels of 14.14, 7.02 and 5.51% respectively. From this, it can be easily seen that the *BV* is inversely related to the protein level. Moreover, the dry matter intake for these rations, being 940.1, 340.3 and 661.9 gms. daily, may be another factor in causing the differences in the values secured.

The combination of 1 part of lucerne hay and 3 parts of sunflower silage with a protein level of 9.31 had yielded a value of 62, and the combination of 1 part of lucerne hay with 3 parts of maize silage a value of 81. The values obtained from the combinations were higher than the arithmetic mean. This would be mainly due to the favourable supplementing effect of the proteins tested.

In 1933, the same author determined with ewes and lambs the nutritive value of lucerne leaves, lucerne stems and the whole hay of three cuttings. The sequence of feeding for each cutting was: (1) nearly N-free rations, (2) stems of lucerne hay, (3) whole lucerne hay, (4) leaves of lucerne hay, and (5) nearly N-free rations.

The nearly N-free mixture contained 460 parts of cut mature Albit wheat straw, 250 parts of cane sugar, 250 parts of powdered maize starch, 10 parts of calcium carbonate and 10 parts of sodium chloride. In addition, each lamb was fed daily 30 gms. of mixed oil consisting of 1 part of cod liver oil and 3 parts of raw linseed oil.

The level of crude protein in the dry matter of stems, whole hay and leaf rations was approximately 8, 13 and 18.5%. The percentage of the total di-

gestible nutrients for the 3 cuttings averaged 41.55, 48.43 and 57.82 for stems, whole hay and leaves respectively. In the same order the nutritive ratios were 1:8.96; 1:3.96 and 1:2.89. The weighted average biological values from the three cuttings were (1) stems 64, (2) whole hay 51, and (3) leaves 41. The variation in the biological values secured is attributed to differences in protein concentrations.

In comparing the nutritive value of lucerne hay protein with that furnished by clover hay, TURK *et al.* (1934) found no significant differences in experiments with lambs. The lucerne hay and clover hay were fed as the only source of protein and each of these hays was also fed with maize grain. Supplements of maize starch, cane sugar, cellulose, maize oil and minerals were added so that the protein contents were about 10%. Furthermore, a low protein ration was fed with 1.92% crude protein. The low-N ration was fed at the beginning and at the end of the experimental periods. The average biological values were 81 for clover protein, 79 for lucerne protein, 80 for the protein in the combination of clover and maize and 77 for the protein in the combination of lucerne and maize.

TURK *et al.* concluded that the difference in protein levels between their experiments and those of SOTOLA for the lucerne hay, might explain the difference in biological values obtained by the two authors.

SMUTS *et al.* (1938) studied the *BV* of lucerne hay and of lucerne hay supplemented with cystine in sheep because it was thought that lucerne hay is deficient in this amino acid. Their experiments were performed at the level of 8% lucerne hay protein and 8% lucerne hay protein supplemented with cystine. The other ingredients of the rations were cod liver oil, dextrinized starch, salt, bone ash and agar. The experiments began and ended with N-free periods. The average *BV* of lucerne protein was found to be 60. The authors concluded that the addition of cystine to lucerne had no effect on the utilization of lucerne protein because when supplementing with cystine the *BV* was still lower (58). This result may be due to the synthesis of cystine by rumen microorganisms in the unsupplemented ration.

MILLER *et al.* (1939) conducted N-balance experiments with growing lambs and they studied the *BV* of protein in four rations. These rations were: (1) maize and lucerne hay, (2) maize and a mixture of two-thirds lucerne hay and one third timothy hay, (3) maize, a mixture of one-third lucerne hay and two-thirds timothy hay, and soybean-oil meal (4) maize, timothy hay and soybean-oil meal. The roughage made up 50% by weight of the total ration and the other ingredients were used in such amounts as were necessary to standardize each ration at 10% protein and at the same energy and mineral content. Average values of *EN* and *MFN* (0.037 gms. of *EN* per kg. of body weight and 0.55 gm. of *MFN* per 100 gms. of dry matter intake) were used in the calculation of the biological values. The average biological values estimated for the four rations were 59, 60, 64 and 62 respectively.

In concluding this chapter, we may say that very few experiments have been performed with ruminants for determining the *BV* of proteins in roughages. More investigations in this field are highly desirable. From the above mentioned trials, it may be concluded that the *BV* of proteins varies inversely with the protein concentration in the diet. For example, SOTOLA (1930, 1933) reported biological values of 56 and 51 respectively for lucerne protein at a protein level of about 14% in dry matter, while at the level of 10%, TURK *et al.* (1934) found

the same protein to have a *BV* of 79. It seems to us, however, that this cannot be the only reason for the large differences observed. The energy intake may be another reason for the wide variation. Moreover, at protein levels of 8% and 10%, SMUTS *et al.* (1938) and MILLER *et al.* (1939) obtained low biological values of 60 and 59 respectively for the lucerne protein. In our opinion the technique of experimentation might be of great importance in determining these values. For a fair comparison, the level of protein, the energy intake and the dry matter intake should be standardized as far as possible.

PART II. EXPERIMENTAL

CHAPTER VII

MATERIALS AND METHODS

The main purpose of this study was to examine the biological value of proteins for sheep in grass hays in the Netherlands. This was performed in nine experiments. The hay was given together with a concentrate mixture low in protein and rich in starch. Some of the rations contained very little hay and hay protein, others, contained moderate amounts. The nitrogen metabolism was studied by determining the protein ingested and the quantities excreted in faeces and urine. From these data the biological values were calculated.

A. EXPERIMENTAL ANIMALS

The investigations were carried out with male lambs of the Texel breed. In the first four experiments three wethers of about one year old were used. They were bought at the market in February 1959. These lambs will be called nos. 1, 2 and 3. After finishing the first two experiments (SI and SII) lamb no. 1 became ill. It showed signs of edema. On examination of its faeces we found that they contained a large amount of worm eggs from various species. The faeces of the other two lambs also contained worm eggs but to a smaller amount. For detecting the worm eggs in the faeces the method of LIESS as mentioned in the book of MALKMUS OPPERMAN (1937) was used. In a centrifuge tube a small quantity of faeces was mixed with water. An equal volume of a concentrated sugar solution (50%) was added. The mixture was centrifuged causing the eggs to float on top of the solution. With a glass rod some substance of the surface was transferred to a microscopic slide for examination.

It is possible that also the protein-poor diet was conducive to the development of the edema.

All three lambs were treated with "Minel"powders, a mixture of phenothiazine and hexachlorethane. When the lambs had recovered and the eggs had disappeared from their faeces, the haemoglobin content of their blood was estimated. Lamb no. 1 showed anaemia. Therefore it was discarded from further investigation.

For experiments SIII and SIV only the remaining two lambs were used. After the fourth experiment (SIV) the lambs were sold and the metabolism crates were thoroughly cleaned.

For the following five experiments another group of four male lambs was selected from the herd of the Experimental Farm belonging to the Laboratory

of Animal Physiology at Wageningen. They were born in March and April 1959. These lambs were not castrated. To prevent complications during the experiments their faeces were tested for worm eggs before starting the trials. It appeared that one of them had many eggs it its faeces. Moreover, its food consumption was poor. Therefore these experiments were carried out with the other three lambs only. They will be called nos. 4, 5 and 6.

In the second part (second subperiod) of the fifth experiment (SV) lamb no. 5 left much of the food. It also developed diarrhea. Therefore the results of that period for this lamb were not used in the calculations. During the preliminary period of experiment SVII lamb no. 6 suddenly lost its appetite and went off feed; it was also excluded from this experiment.

Before conducting the last two experiments (SVIII and SIX) the animals were kept outdoors on pasture for about 6 weeks for some exercise and, if necessary, to replenish loss of protein.

Prior to the initiation of the investigations all the lambs were sheared and washed thoroughly with warm water and chlorine. During the experiments the lambs were housed in metabolism crates. They were harnessed to facilitate collection of faeces and urine. The faeces were collected in weighed rubber bags. The urine was collected through rubber funnels attached to the harnesses. These funnels were connected to plastic or rubber tubing which emptied in weighed glass bottles.

B. Diets

The hays used for these experiments were harvested from pastures situated near Wageningen. Data concerning the hay making are collected in table 9.

Table 9. Data on the hay making

Hay tested	Cut	Hanged over frame-driers	Brought indoors
Early spring hay (1958)	24–5–1958	29–5–1958	11–6–1958
Early spring hay (1959)	14–5–1959	19–5–1959	1–6–1959
Late spring hay (1959)	30–5–1959	1–6–1959	12–6–1959
Autumn hay (1959)	20–8–1959	24–8–1959	6–9–1959

During hay making the weather conditions were very good, although some rain had fallen during the making of the early spring hay 1958. It was dry when the other three hays were made. In all four cases the temperature indoors during heating was always below 30 °C.

The analysis of the hays are given in table 12. In table 10 some data concerning the botanical composition are presented.

As was shown by earlier investigators it is difficult to compose a diet which is free from protein and at the same time palatable to the animals. Therefore we used another scheme for investigating the biological value of hay proteins. In this scheme we gave low protein diets to the animals in one experiment, and diets with a moderate level in a second. After some attempts we succeeded in composing diets which were low and moderate in protein giving no feed residues.

Diets low in protein were given in experiments SI, SIII and SV. They were successively followed by experiments SII, SIV and SVI, which contained higher amounts of protein. SVII was nearly a repetition of SV, only the amount of

TABLE 10. Botanical composition of spring hays

Quality of grass and degree	Early spring hay (1958) (%)	Early spring hay (1959) (%)	Late spring hay (1959) (%)
A. Good grasses (8–10)	72	72	72
B. Medium „ (5– 7)	21	23	23
C. Poor „ (0– 4)	4	3	2
D. Herbs (0– 4)	3	2	3
A. Lolium perenne L.	14	12	12
Festuca pratensis Huds.	–	+	1
Phleum pratense L.	2	1	2
Poa pratensis L.	1	1	2
Poa trivialis L.	55	58	55
B. Alopecurus prat. L.	7	7	6
Cynosurus cristatus L.	3	1	2
Dactylis glomerata L.	7	1	2
Agrostis stolonifera L.	2	1	+
Holcus lanatus L.	2	13	12
C. Anthoxanthum odoratum L.	4	2	2
D. Ranunculus repens L.	1	1	2

+ Means present in less than 0.5%.

cellulose was reduced. The first four experiments were carried out with the early spring hay cut on 24/5/1958, the next three experiments, SV, SVI and SVII, with the early spring hay cut on 14/5/1959 (table 9).

Experiments SVIII and SIX were designed mainly to compare the biological values of two other hays harvested in two different seasons (autumn and late spring) of the same year (1959) (table 9). In both experiments, diets moderate in hay protein were given. The hay examined in SVIII was the autumn hay, SIX was performed with the late spring hay.

The rations always consisted of the hay to be tested and a concentrate mixture. The composition of these rations and the amounts offered to the animals are presented in table 11 (p. 56–57).

In order to learn how many *SE* were ingested by the experimental animals we had calculated the *SE* (starch equivalent) and *dcp* (digestible crude protein) content for every component of the various rations.

For the hays these computations were done after the principle of BROUWER and DIJKSTRA as practised by DIJKSTRA (1951) and DIJKSTRA *et al.* (1955). These formulae which give the *SE* and the *dcp* in percentages of the dry matter, are as follows:

$$a.\ SE/DM = -1.666\ (y' - 32) - 1.004\ (m' - 9) + 38.06,$$
$$b.\ dcp/DM = -0.809\ (x' - 12) + 0.035\ (m' - 9) + 6.52,$$

where x', y' and m' represent the percentages of crude protein, crude fibre and ash respectively in the dry matter. The results are collected in table 12 (p. 56–57).

In calculating the *SE* and the *dcp* content of the other ingredients, the tables of LEIGNES BAKHOVEN (1951) were consulted. Following HVIDSTEN (1946) we assumed the *SE* of the cellulose to be 56. HVIDSTEN came to the conclusion that common, bleached sulphite cellulose contains 0.8 Scandinavian feed units per

kg. of dry cellulose (with 90% of dry matter). Thus, the starch equivalent would be 0.7 x 0.8 = 0.56 per kg. dry cellulose (with 90% dry matter). As the dry matter content of the cellulose used in our experiments was only little higher than 90% we stuck to the value of 0.56 per kg. of fresh material.

Great care was taken to ensure that each animal received the same quantity of feed every day in the preliminary as well as in the experimental period. In the case of hays, where the finest material always sinks to the bottom, special precautions had to be taken. The hays were chopped and then divided into three fractions (coarse, middle and fine) by passing them through sieves of 1 cm. and 0.5 cm. diameter. Of each fraction aliquote amounts were weighed for the daily rations. This procedure also improved considerably the representativeness of the samples which were taken from all three fractions and were also mixed in aliquote parts.

The concentrates were given as a mixture. The daily amounts were weighed in paper bags. The cellulose was also weighed in separate bags, and added to the concentrate mixture only at the time of feeding.

The diets were composed in such a way that the net energy was somewhat greater than the maintenance requirement, in the low-protein diets as well as in the moderate-protein ones. Additional minerals were supplied in the rations. These minerals consisted of a mixture of equal amounts of sodium chloride, potassium chloride and di-calcium phosphate. In the calculations, for convenience sake, it was assumed that they were free from water and that they did not contain nitrogenous substances. No vitamins were added because we assumed that the diets contained sufficient amounts of them, or that the reserves would be satisfactory for the time of the experiments.

As the weights of the animals in every experiment were very similar, we considered their needs of *SE* and *dcp* to be the same (see table 11).

With the aid of FREDERIKSEN's formulae for the daily requirements of *SE* and *dcp* for the maintenance of dairy cows (1931), we calculated these values for our animals. As we were using sheep instead of cows some extensions of the formulae were necessary; they are explained in the following way:

According to FREDERIKSEN's formulae:

$$\text{kg. } SE \text{ for maintenance of a cow} = \frac{V}{300} + 1,$$

$$\text{gms. } dcp \text{ „ „ „ „ „} = 0.7\,V,$$

in which V = the body weight in kilograms.

Thus for a cow of 500 kgs. live weight:

$$SE = \frac{500}{300} + 1 \quad = 2.67 \text{ kgs.}$$

and

$$dcp = 0.7 \times 500 = 350 \text{ gms.}$$

According to KLEIBER (1947) the $^3/_4$ power of body weight is recommended as the representative of metabolic body size, so that for a sheep of 35 kgs. body weight:

$$SE = \left(\frac{35}{500}\right)^{3/4} \times 2670 = 363.4 \text{ gms.}$$

and

$$dcp = \left(\frac{35}{500}\right)^{3/4} \times 350 = 47.6 \text{ gms.}$$

TABLE 11. Feed given to the animals with the dry matter, crude protein, starch equivalent and dige

Hay tested	Experiment	Ingredients of rations (gms.)						
		Hay	Potato starch	Maize starch	Cellulose	Carrots meal	Minerals	T
Early Spring Hay 28–5–1958	SI 7–3–59 21–3–59	200.0	220.5	–	130.5	85.5	13.5	6
	SII 2–4–59 16–4–59	400.0	171.5	–	101.5	66.5	10.5	7
	SIII 4–6–59 18–6–59	100.0	260.0	–	130.0	80.0	15.0	5
	SIV 2–7–59 16–7–59	300.0	210.0	–	100.0	80.0	15.0	7
Early Spring Hay 14–5–1959	SV 24–8–59 7–9–59	100.0	–	250.0	250.0	50.0	15.0	6
	SVI 24–9–59 8–10–59	300.0	–	250.0	–	50.0	15.0	6
	SVII 22–10–59 5–11–59	100.0	–	250.0	200.0	50.0	15.0	6
Autumn hay 20–8–1959	SVIII 3–3–60 17–3–60	300.0	–	300.0	–	50.0	15.0	6
Late spring hay 30–5–1959	SIX 29–3–60 12–4–60	400.0	–	250.0	–	50.0	15.0	7

* Starch equivalent and digestible crude protein requirements as calculated by FREDERIKSEN and KI

TABLE 12. Analysis of the hays with their starch equivalent and dig

Hay tested	Sample no.	% of dry matt in fresh materi
Early spring hay (1958)	1821	84.11
Early spring hay (1959)	2015	86.23
Late spring hay (1959)	2016	87.29
Autumn hay (1959)	3012	83.24

de protein

Offered (gms.)				Crude protein in dry matter (%)	Dig. crude prot. per 100 gms. starch equivalent	Dig. crude prot. in dry matter (%)
Dry matter	Crude protein	Starch equivalent	Digestible crude protein			
570.48	43.23	371.9 296.3*	21.4 38.8*	7.6	5.8	3.8
654.90	64.09	377.0 304.5*	37.7 39.9*	9.8	10.0	5.9
509.13	21.11	363.6 299.0*	12.5 39.2*	4.2	3.4	2.5
615.61	51.38	379.0 303.2*	29.7 39.8*	8.4	7.8	4.8
589.10	18.73	427.5 366.3*	10.5 48.0*	3.2	2.5	2.1
542.56	46.82	376.3 355.6*	26.8 46.6*	8.6	7.1	4.9
549.25	19.05	399.5 363.4*	10.5 47.6*	3.5	2.6	2.0
585.93	60.55	395.4 399.1*	35.5 52.3*	10.3	9.0	6.1
640.82	46.94	410.4 401.6*	25.0 52.6*	7.3	6.1	3.9

mulae according to the actual body weights of the lambs.

e protein content on dry matter basis

Percentage in the dry matter				
rude protein (%)	Crude fibre (%)	Ash (%)	Starch equivalent	Dig. crude protein (%)
16.51	27.65	11.64	42.66	10.26
15.58	23.69	9.45	51.45	9.44
11.94	26.27	8.71	47.90	6.46
20.23	27.23	11.05	43.95	13.25

The figures obtained by the above formulae for *SE* and *dcp* are somewhat higher than the maintenance allowances as recommended by the CVB in the Netherlands (1959). The CVB (Centraal Veevoederbureau) is the board which deals with animal feeding and which publishes recommendations about feeding-standards to be applied under conditions prevailing in the Netherlands. For a sheep of 50–60 kgs. live weight, 450 gms. *SE* and 55 gms. *dcp* is advised for maintenance. According to these standards a sheep of 35 kgs. would need 285 gms. *SE* and 35 gms. *dcp*. These figures are lower than those obtained by FREDERIKSEN's formulae when applied to sheep.

In table 11 it may be seen that the rations contained somewhat more, sometimes even considerably more *SE* than calculated for maintenance, however with one exception, namely the autumn hay 1959. The reason for this is that this hay, although being rich in protein, had a high crude fibre content.

The starch equivalent as given to the animals, ranged from 364 to 428 gms., and the digestible crude protein from 10 to 38 gms. for the various rations.

Table 11 indicates that protein levels are somewhat higher when relating the *dcp* to the *SE* instead of to the dry matter. This is especially the case in rations containing a large amount of roughage. These differences are caused by the higher and varying percentages of crude fibre. Therefore it is better to relate the *dcp* to *SE*, rather than to the dry matter.

C. DETAILS OF THE EXPERIMENTS

In every experiment there was one experimental period of a fortnight subdivided into two successive subperiods of one week each (subperiod *a* and subperiod *b*). The experimental periods were preceded by preliminary periods of about eleven days. The preliminary periods were started after the animals had been accustomed to the rations and had been consuming the whole daily rations for at least four days.

The food was given to the animals in two portions. The first half at 16.30 in the evening and the second half at 8.00 o'clock the next morning. First, the concentrate mixture was given after moistening with an equal amount of water. Then drinking water was offered *ad libitum*. Finally the hay was given. The feed residues were collected at the end of each subperiod at the same hour (16.30). They were weighed, air-dried and ground. The lambs were weighed at 10.30 a.m., at weekly intervals (at the beginning and the end of the subperiods).

We started to collect the urine at 9.00 a.m., $16\frac{1}{2}$ hours after the first part of the experimental feed was given, and we stopped the collection at 9.00 a.m., seven days later. The faeces however were collected $40\frac{1}{2}$ hours after the first part of the experimental feed was given; this was done because MØLLGAARD in 1929 had stated that with a relatively short preliminary period, more accurate results were obtained when the faeces were not collected too soon after the first experimental feed had been given. Of course this holds true only if the same ration is fed for two more days after the experimental period is ended, which was actually practised in our experiments.

The collected urine and faeces were weighed daily during the experimental periods with an accuracy of ± 1 gm. Both were preserved; the urine was acidified with HCl and then kept in a refrigerator at about 5°C; the faeces were stored with formaline at $\pm(-20)$°C.

D. Collection and analysis of samples

1. *Hay sampling*

The hay, as already mentioned, was divided into three fractions: coarse, middle and fine. Day-rations were prepared by mixing aliquote parts of these fractions in paper bags. Before adjusting the weight of every fraction, two representative samples were taken from them for analysis and collected in two containers. In this way three duplicate samples were obtained: coarse, middle and fine A and coarse, middle and fine B. From each of the A and the B samples 200 gms. were taken and air-dried at 60–70°C for about 16 hours. These samples were weighed and ground after keeping them for about four hours at room temperature. According to the percentages of the three fractions and of the air-dry matter found the three samples were again mixed. In this manner two samples resulted, both representing the hay as given to the animals. Dry matter and crude protein were determined in these hay samples. In the preliminary periods sampling however was left out.

2. *Sampling of the concentrate mixture*

Representative samples were taken from every ingredient before they were mixed. In addition a sample was taken from the mixture at the moment when the daily rations were weighed for the experimental periods. This sample was used as a check. In the cases where cellulose was included in the ration, a separate sample was taken from it at the time when it was weighed for the experimental periods. Cellulose was mixed with the other concentrates only at the time of feeding.

3. *Sampling of urine and faeces*

The urine of each lamb was collected for a subperiod in large plastic containers. An amount of 50 ml. (55 gms.) HCl 1:1 was poured into the containers before collecting the urine. The collected urine was checked every day for its acidity. Whenever the urine became alkaline it was acidified again by adding a second portion of 50 ml. HCl 1:1, or more if necessary. The urine collected in every subperiod was thoroughly mixed. Two representative samples were drawn from it for nitrogen determination. The figures obtained were corrected for the addition of HCl.

The faeces of the experimental periods were collected in big containers, one for each subperiod. Formaline was added in portions of 15 ml. (16 gms.) for each container. The first 10 ml. of formaline were put in the container at the beginning, the other 5 ml. were added after the third day. At the end of every subperiod the contents of the containers were mixed thoroughly. Samples were taken from them for the determination of dry matter, crude protein and digestible crude protein. The results were corrected for the addition of the formaline. In this case the correction is more complicated than for the addition of HCl to urine because the formaline upon drying together with the faeces gives a residue. This residue is included in the weight of the dry matter.

The correction formula can be explained as follows:

suppose that the quantity of faeces is m gms. and 1 gm. of faeces contains a gms. of dry matter, then the dry matter in the faeces is ma gms.

When the amount of formaline added is p gms. and the dry matter in 1 gm.

formaline is supposed to be 0.4 gms., the amount of dry matter in the formaline is $0.4\,p$.

If the percentage of the determined dry matter is a',

the total dry matter is

$$(m + p)a' \text{ gms.}$$

As

$$ma = (m + p)a' - 0.4\,p,$$

the dry matter in the faeces before the addition of the formaline can be calculated:

$$a = \frac{(m + p)a' - 0.4\,p}{m} \text{ gms.}$$

Further samples of 1 kg. fresh faeces were weighed, air-dried and ground to be used in case of need.

4. *Chemical determinations*

All nitrogen determinations were made by the official Kjeldahl method. Protein was calculated as N $\times$ 6.25. The digestible crude protein was determined by the pepsin-HCl method (Rijkslandbouwproefstation, Maastricht, 1954). To determine the dry matter, the materials were dried as usual at $\pm$103°C. All chemical determinations were done in triplicate.

CHAPTER VIII

RESULTS AND DISCUSSION

The diets – In determining the biological value of proteins, mostly growing rats have been used so that the figures for biological values in the literature mainly refer to maintenance plus growth.

As the sheep in our experiments did not eat large quantities of the experimental rations, growth was only small so that most of the feed was used for maintenance alone. We are, however, convinced that this does not impair the value of the results because, in animal feeding, hay is in the first place used for maintenance. It is therefore important to know the biological value of the hay protein for maintenance, no other functions being superimposed. However, it should be kept in mind that in sheep the wool growth does not stop in animals on maintenance rations. A zero balance should therefore be explained as a negative one as far as the living tissues are concerned. Even a small positive balance does not prove that protein is gained in the animal organs.

Although the wool growth in an animal on maintenance ration undoubtedly will be somewhat lower than in a full-fed animal, we feel that on maintenance ration, the daily quantity of newly-built wool protein will not fall down to below some 4.5 gms. per day (MITCHELL *et al.*, 1928). Protein balances below 4.5 gms., in our opinion, must therefore be considered as negative as far as the living tissues are concerned. As a matter of fact, in our experiments, the protein balances only once rose to + 9 gms. a day, so that by far the greater part of the feed protein was used for maintenance of the tissues and, in addition, for the production of some wool protein.

As already has been explained in chapter VII the hays investigated were:

1) Early spring hay (1958): SI, SII, SIII, SIV
2) Early spring hay (1959): SV, SVI, SVII
3) Autumn hay (1959): SVIII
4) Late spring hay (1959): SIX.

In order to examine whether the energy provision of the animals was sufficient the starch value of the rations was calculated with the aid of the methods mentioned in chapter VII. These values were compared with the requirements calculated with the formulae given in the same chapter. From table 11 it may be seen that in all the experiments the starch value of the rations as given to the animals always exceeded the required ones. One exception to this generalization was SVIII. There the calculated starch value was 4 gms. lower than required. We do not consider it probable that this has markedly injured our determination of *BV* because the animal had been depleted to some extent in the preceding low protein period. In all experiments the animals recorded gains or losses in body weights within small limits. In the protein-poor experiments the weekly gains ranged from −2.1 to +0.3 kgs.; in the rations with moderate protein the limits were −0.7 to +1.1 kgs. This means that the rations were well adjusted to the maintenance level and that, with perhaps some few exceptions, the starch values were quite sufficient to maintain the body weights of the animals during these experiments.

As regards the *protein* it may be seen from tables 11 and 13 that, besides the protein of the hay, there was some protein in the carrots meal which had been added to the diet in order to make the latter acceptable to the sheep. The hay protein, however, constituted the major part. Even in the protein-poor control diets (SI, SIII, SV, SVII) the hay proteins ranged from 67.9 to 71.8% of the total amount. In the experimental diets with higher protein content (SII, SIV, SVI, SVIII, SIX) they ranged from 87.0 to 92.4%. It is therefore clear that the latter experiments are of primary importance.

The data secured in the experiments are presented in table 13 (feed analysis), table 14 (excreta and their analysis), tables 15, 16 and 17 (digestion coefficients and biological values).

TABLE 13. Feed analysis (%)

Experiment	Hay		Potato starch		Maize starch		Cellulose		Carrots meal	
	Dry matter	Crude protein in dry matter	Dry matter	Crude protein in dry matter	Dry matter	Crude protein in dry matter	Dry matter	Crude protein in dry matter	Dry matter	Crude protein in dry matter
SI	89.20	16.46	85.48	0.44	–	–	93.27	0.34	79.98	18.47
SII	88.57	16.51	83.51	0.24	–	–	91.19	0.35	81.72	9.09
SIII	88.68	16.45	83.00	0.08	–	–	92.59	0.23	86.60	8.76
SIV	88.31	16.87	83.21	0.19	–	–	91.66	0.32	86.60	8.76
SV	86.95	15.46	–	–	86.53	0.22	91.90	0.11	82.17	11.10
SVI	89.42	15.50	–	–	87.29	0.31	–	–	82.17	11.10
SVII	88.66	15.43	–	–	87.37	0.25	92.54	0.11	84.18	10.98
SVIII	87.68	21.28	–	–	88.15	0.33	–	–	86.87	8.55
SIX	90.50	11.74	–	–	88.15	0.33	–	–	86.87	8.55

Digestibility – The digestibility of the dry matter ranged from 60 to 80%. It was lowest in SIII (about 60%) and highest in SVI (about 80%).

In table 18 the average digestibility of the dry matter in the experimental rations SII, SIV and SVI is compared with that in the corresponding control rations SI, SIII, SV – SVII.

Although the protein-poor rations contained only little crude fibre, the digestion coefficients of the dry matter were on the average some units lower than those of the rations to which the hays had been added. This means that there was a certain degree of "digestibility depression" in the protein-poor rations, obviously caused by lack of protein.

As might be expected the apparent digestibility of the crude protein was much more variable, ranging from negative to about +52%. A large part of this variation is related to the protein content of the diet; the digestion coefficients were lowest in the protein-poor control diets, used in SI, SIII, SV, SVII (being +19.2, −1.7, −22.9 and +6.4% respectively) and much higher in the experimental diets of SII, SIV, SVI, SVIII, SIX (+37.7, +36.0, +50.4, +51.7 and +42.2% respectively).

It is well known that the digestibility depression of the protein in protein-poor rations is largely due to the metabolic faecal protein (chapter III). Figures for this metabolic faecal protein were obtained by us in two ways: firstly as an average value calculated from the literature (3.438 gm. per 100 gms. of ingested dry matter, chapter III), and secondly from determinations of the faecal protein soluble in pepsin-HCl (table 14). In some experiments the calculated values were lower than those determined by the pepsin-HCl method (SI, SII, SIV). In all the other experiments the calculated values were higher, especially in SIX. In this experiment the pepsin-HCl method gave a value only $^2/_3$ of the calculated one (table 15).

It is obvious that the true digestion coefficients obtained with the two figures for metabolic faecal protein also must show considerable differences in the same sense, the true digestion coefficient becoming higher with higher figures for metabolic faecal protein. The highest values for the true digestion coefficients were obtained in SVII (metabolic faecal protein calculated; protein-poor ration; lamb no. 4), two coefficients being even greater than 100. In this case the calculated value for metabolic faecal protein is higher than the total amount of protein in the faeces, so that, at least in this case, the factor for the calculation of metabolic faecal protein (3.438 gm. per 100 gms. of ingested dry matter) must have been too high.

We now compare the digestibility of the proteins in the rations as secured experimentally with those obtained by the computations with the formulae b and c of HUISMAN (1946). These formulae are as follows:

$$y = 0.837\,x - 3.13 \text{ (hay)} \quad \text{b)}$$
$$y = 0.923\,x - 3.90 \text{ (from KELLNER's tables)} \quad \text{c)}$$

where y is the digestion coefficient and x is the crude protein in organic matter. The results on organic matter basis are shown in table 19.

From table 19 it may be observed that the results obtained experimentally are often different from those computed by HUISMAN's formulae. However in most instances the agreement is very good, except SI and SII.

TABLE 14. Excreta and their analysis

Experiment	Lamb	Main period	Faeces				Urine	
			Amount (gms.)	Dry matter (%)	Crude protein in dry matter (%)	Metab. faec. protein (Pepsin–HCl) (%)	Amount (gms.)	'Crude protein' (6.25 N) (%)
SI	1	a	715.7	22.93	24.03	15.87	651.4	2.69
		b	600.9	23.77	22.76	14.81	716.1	1.85
	2	a	825.3	25.91	16.40	11.85	622.3	1.88
		b	532.3	31.12	19.09	12.44	586.9	1.69
	3	a	670.4	26.47	19.91	12.39	539.3	1.98
		b	664.0	24.96	20.19	12.66	575.7	1.70
SII	1	a	679.1	25.76	22.63	15.14	934.7	1.85
		b	682.3	27.29	20.85	12.83	826.6	2.46
	2	a	529.6	32.50	21.26	12.68	647.9	3.12
		b	468.4	33.63	22.12	13.41	679.7	2.77
	3	a	959.3	20.45	21.08	13.11	642.3	2.04
		b	926.4	19.37	24.06	15.18	632.9	2.23
SIII	2	a	534.1	37.71	10.87	7.96	624.6	0.99
		b	551.4	36.76	10.96	8.08	609.3	1.05
	3	a	1047.4	19.06	11.07	7.82	549.4	1.36
		b	825.0	25.28	10.56	7.44	628.0	1.28
SIV	2	a	689.7	28.23	17.07	12.08	751.0	1.27
		b	598.7	30.30	18.42	12.01	715.0	1.33
	3	a	851.4	23.53	16.23	12.07	928.7	1.06
		b	828.6	23.78	16.44	10.56	875.1	1.17
SV	4	a	466.4	33.13	12.71	9.15	324.6	2.44
		b	349.1	33.72	14.71	10.85	367.0	2.50
	5	a	542.0	24.56	18.53	14.90	399.5	2.65
	6	a	428.4	31.00	17.87	13.68	276.0	2.87
		b	284.4	39.50	15.80	11.32	397.4	2.46
SVI	4	a	325.7	33.93	21.31	11.32	606.7	3.07
		b	373.9	33.30	20.36	9.91	670.6	2.96
	5	a	274.1	37.33	21.35	10.02	672.3	3.59
		b	323.9	34.74	21.50	10.19	558.4	4.33
	6	a	282.7	39.91	20.77	11.02	515.6	3.69
		b	257.3	39.50	20.71	11.27	470.3	4.18
SVII	4	a	512.4	37.10	8.92	6.42	1294.6	0.75
		b	528.0	36.83	8.20	5.59	994.1	0.96
	5	a	435.4	31.69	13.95	9.56	1225.3	0.69
		b	354.1	31.77	16.02	10.20	997.4	0.97
SVIII	4	a	407.0	35.52	20.02	11.37	1160.1	2.43
		b	405.7	33.87	17.80	11.60	1368.9	1.98
	5	a	405.4	36.00	19.67	9.56	1169.4	2.67
		b	457.3	31.93	20.20	11.21	783.7	4.02
	6	a	396.6	39.40	20.43	10.18	665.4	4.07
		b	441.0	31.40	22.68	11.97	694.1	3.70
SIX	4	a	508.6	33.13	16.06	8.78	641.6	2.14
		b	517.6	32.23	17.06	9.15	575.4	2.08
	5	a	436.4	36.90	15.96	8.24	606.4	2.27
		b	392.7	38.60	17.07	7.77	636.9	2.14
	6	a	407.0	37.86	17.43	8.48	619.0	2.11
		b	460.6	36.47	17.16	9.38	633.7	2.20

TABLE 15. Digestion coefficients of dry matter and crude protein

Experiment	Lamb	Main period	Dry matter			Crude protein								
			Ingested (gms.)	Faeces (gms.)	Digestion coefficient (%)	Ingested (gms.)	Faeces (gms.)	Metabolic faecal protein (gms.)		Absorbed protein (gms.)		Apparent digestion coefficient (%)	True digestion coefficient (%)	
								Calculated*	Pepsin-HCl method	Calculated*	Pepsin-HCl method		Calculated*	Pepsin-HCl method
SI	1	a	563.34	164.11	70.9	42.68	39.44	19.37	26.04	22.61	29.28	7.6	53.0	68.6
		b	564.01	142.82	74.7	42.59	32.51	19.39	21.15	29.47	31.23	23.7	69.2	73.3
		average	*563.68*	*153.46*	*72.8*	*42.64*	*35.98*	*19.38*	*23.60*	*26.04*	*30.26*	*15.6*	*61.1*	*71.0*
	2	a	569.72	213.83	62.5	43.23	35.07	19.59	25.34	27.75	33.50	18.9	64.2	77.5
		b	568.54	165.65	70.9	43.23	31.62	19.55	20.61	31.16	32.22	26.8	72.1	74.5
		average	*569.13*	*189.74*	*66.7*	*43.23*	*33.34*	*19.57*	*22.98*	*29.46*	*32.86*	*22.8*	*68.1*	*76.0*
	3	a	566.11	177.46	68.6	42.76	35.33	19.46	21.99	26.89	29.42	17.4	62.9	68.8
		b	562.18	165.73	70.5	42.39	33.46	19.33	20.98	28.26	29.91	21.1	66.7	70.6
		average	*564.14*	*171.60*	*69.6*	*42.58*	*34.40*	*19.40*	*21.48*	*27.58*	*29.66*	*19.2*	*64.8*	*69.7*
SII	1	a	643.67	174.95	72.8	62.92	39.59	22.13	26.49	45.46	49.82	37.1	72.3	79.2
		b	642.42	186.20	71.0	62.79	38.82	22.09	23.89	46.06	47.86	38.2	73.4	76.2
		average	*643.04*	*180.58*	*71.9*	*62.86*	*39.20*	*22.11*	*25.19*	*45.76*	*48.84*	*37.6*	*72.8*	*77.7*
	2	a	647.99	172.11	73.4	63.50	36.59	22.28	21.82	49.19	48.73	42.4	77.5	76.7
		b	648.15	157.53	75.7	63.41	34.85	22.28	21.12	50.84	49.68	45.0	80.2	78.3
		average	*648.07*	*164.82*	*74.6*	*63.46*	*35.72*	*22.28*	*21.47*	*50.02*	*49.20*	*43.7*	*78.8*	*77.5*
	3	a	636.16	196.17	69.2	61.93	41.35	21.87	25.72	42.45	46.30	33.2	68.5	74.8
		b	637.58	179.45	71.8	62.10	43.18	21.92	27.24	40.84	46.16	30.5	65.8	74.3
		average	*636.87*	*187.81*	*70.5*	*62.02*	*42.26*	*21.90*	*26.48*	*41.64*	*46.23*	*31.8*	*67.2*	*74.6*
SIII	2	a	509.13	201.42	60.4	21.11	21.89	17.50	16.03	16.72	15.25	–3.7	79.2	72.2
		b	509.13	202.71	60.2	21.11	22.22	17.50	16.38	16.39	15.27	–1.1	77.6	72.3
		average	*509.13*	*202.06*	*60.3*	*21.11*	*22.06*	*17.50*	*16.20*	*16.56*	*15.26*	*–2.4*	*78.4*	*72.2*
	3	a	509.13	199.64	60.8	21.11	22.10	17.50	15.61	16.51	14.62	–1.0	78.2	69.3
		b	509.13	208.56	59.0	21.11	22.02	17.50	15.52	16.59	14.61	–0.9	78.6	69.2
		average	*509.13*	*204.10*	*59.9*	*21.11*	*22.06*	*17.50*	*15.56*	*16.55*	*14.62*	*–1.0*	*78.4*	*69.2*
SIV	2	a	615.61	194.71	68.4	51.38	33.24	21.16	23.52	39.30	41.66	35.3	76.5	81.1
		b	615.61	181.41	70.5	51.38	33.42	21.16	21.79	39.12	39.75	35.0	76.1	77.4
		average	*615.61*	*188.06*	*69.4*	*51.38*	*33.33*	*21.16*	*22.66*	*39.21*	*40.70*	*35.2*	*76.3*	*79.2*
	3	a	615.61	200.34	67.5	51.38	32.52	21.16	24.18	40.02	43.04	36.7	77.9	83.8
		b	615.61	197.03	68.0	51.38	32.39	21.16	20.81	40.15	39.80	37.0	78.1	77.5
		average	*615.61*	*198.68*	*67.8*	*51.38*	*32.46*	*21.16*	*22.50*	*40.08*	*41.42*	*36.8*	*78.0*	*80.6*
SV	4	a	535.78	154.53	71.2	18.06	19.64	18.42	14.14	16.84	12.56	–8.8	93.2	69.5
		b	482.85	117.73	75.6	16.25	17.32	16.60	12.77	15.53	11.70	–6.6	95.6	72.0
		average	*509.32*	*136.13*	*73.4*	*17.16*	*18.48*	*17.51*	*13.46*	*16.18*	*12.13*	*–7.7*	*94.4*	*70.8*
	5	a	487.58	132.79	72.8	17.11	24.61	16.76	19.79	9.26	12.29	–43.8	54.1	71.8
	6	a	528.83	132.81	74.9	17.97	23.73	18.18	18.17	12.42	12.41	–32.0	69.1	69.1

	5	a	542.56	102.34	81.1	46.82	21.85	18.65	10.25	43.62	35.22	53.3	93.2	75.2
		b	542.56	112.51	79.3	46.82	24.19	18.65	11.46	41.28	34.09	48.3	88.2	72.8
		average	*542.56*	*107.42*	*80.2*	*46.82*	*23.02*	*18.65*	*10.86*	*42.45*	*34.66*	*50.8*	*90.7*	*74.0*
	6	a	542.56	112.83	79.2	46.82	23.43	18.65	12.43	42.04	35.82	50.0	89.8	76.5
		b	542.56	101.63	81.3	46.82	21.05	18.65	11.45	44.42	37.22	55.0	94.9	79.5
		average	*542.56*	*107.23*	*80.2*	*46.82*	*22.24*	*18.65*	*11.94*	*43.23*	*36.52*	*52.5*	*92.4*	*78.0*
SVII	4	a	549.25	190.11	65.4	19.05	16.96	18.88	12.21	20.97	14.30	11.0	110.1	75.1
		b	549.25	194.46	64.6	19.05	15.95	18.88	10.87	21.98	13.97	16.3	115.4	73.3
		average	*549.25*	*192.28*	*65.0*	*19.05*	*16.46*	*18.88*	*11.54*	*21.48*	*14.14*	*13.6*	*112.8*	*74.2*
	5	a	549.25	137.99	74.9	19.05	19.25	18.88	13.19	18.68	12.99	–1.0	98.1	68.2
		b	457.62	112.51	75.4	17.67	18.02	15.73	11.48	15.38	11.13	–0.4	87.0	63.0
		average	*503.44*	*125.25*	*75.1*	*18.36*	*18.64*	*17.30*	*12.34*	*17.03*	*12.06*	*–0.7*	*92.6*	*65.6*
SVIII	4	a	581.05	144.57	75.1	60.36	28.94	19.98	16.44	51.40	47.86	52.0	85.2	79.3
		b	585.93	137.41	76.6	60.55	24.46	20.14	15.94	56.20	52.03	59.6	92.8	85.9
		average	*583.49*	*140.99*	*75.8*	*60.46*	*26.70*	*20.06*	*16.19*	*53.80*	*49.94*	*55.8*	*89.0*	*82.6*
	5	a	575.08	145.95	74.6	60.15	28.71	19.77	13.95	51.21	45.39	52.3	85.1	75.5
		b	585.93	146.01	75.1	60.55	29.49	20.14	16.37	51.20	47.43	51.3	84.6	78.3
		average	*580.50*	*145.98*	*74.8*	*60.35*	*29.10*	*19.96*	*15.16*	*51.20*	*46.41*	*51.8*	*84.8*	*76.9*
	6	a	576.16	156.25	72.9	60.01	31.92	19.81	15.91	47.90	44.00	46.8	79.8	73.3
		b	585.93	138.47	76.4	60.55	31.40	20.14	16.57	49.29	45.72	48.1	81.4	75.5
		average	*581.04*	*147.36*	*74.6*	*60.28*	*31.66*	*19.98*	*16.24*	*48.60*	*44.86*	*47.4*	*80.6*	*74.4*
SIX	4	a	640.82	168.49	73.7	46.94	27.06	22.03	14.79	41.91	34.67	42.8	89.3	73.9
		b	640.82	166.81	74.0	46.94	28.46	22.03	15.26	40.51	33.74	38.6	86.3	71.9
		average	*640.82*	*167.65*	*73.8*	*46.94*	*27.76*	*22.03*	*15.02*	*41.21*	*34.20*	*40.7*	*87.8*	*72.9*
	5	a	640.82	161.04	74.9	46.94	25.70	22.03	13.27	43.27	34.51	45.2	92.2	73.5
		b	640.82	151.59	76.3	46.94	25.88	22.03	11.78	43.09	32.84	44.9	91.8	70.0
		average	*640.82*	*156.32*	*75.6*	*46.94*	*25.79*	*22.03*	*12.52*	*43.18*	*33.68*	*45.0*	*92.0*	*71.8*
	6	a	640.82	154.09	76.0	46.94	26.86	22.03	13.07	42.11	33.15	42.4	89.7	70.6
		b	640.82	167.97	73.8	46.94	28.82	22.03	15.76	40.15	33.88	39.4	85.5	72.2
		average	*640.82*	*161.03*	*74.9*	*46.94*	*27.84*	*22.03*	*14.42*	*41.13*	*33.52*	*40.9*	*87.6*	*71.4*

* Metabolic faecal protein = 3.438 gm. per 100 gms. of ingested dry matter.

TABLE 16. Biological values of the hays calculated by two methods

Experiment	Lamb	Main period	Weight of Lamb (kgs.)	Protein ingested (gms.)	Faecal Protein (gms.)			'Urine protein' (6.25 N) (gms.)		Protein balance (gms.)	Biological values (%)	
					Total amount	Metabolic faecal protein [1]		Total amount	Endogenous [2]		Thomas–Mitchell method [3]	
						Calculated	Pepsin–HCl method				3a	3b
SI	1	a	27.84	42.68	39.44	19.37	26.04	17.52	6.43	−14.28	51.0	62.1
		b	28.19	42.59	32.51	19.39	21.15	13.25	6.51	− 3.17	77.1	78.4
		average	*28.02*	*42.64*	*35.98*	*19.38*	*23.60*	*15.38*	*6.47*	*− 8.72*	*64.0*	*70.2*
	2	a	26.45	43.23	35.07	19.59	25.34	11.70	6.11	− 3.54	79.9	83.3
		b	26.15	43.23	31.62	19.55	20.61	9.92	6.04	+1.69	87.6	88.0
		average	*26.30*	*43.23*	*33.34*	*19.57*	*22.98*	*10.81*	*6.08*	*− 0.92*	*83.8*	*85.6*
	3	a	26.27	42.76	35.33	19.46	21.99	10.68	6.07	− 3.25	82.9	84.3
		b	26.47	42.39	33.46	19.33	20.98	9.79	6.11	− 0.86	87.0	87.7
		average	*26.37*	*42.58*	*34.40*	*19.40*	*21.48*	*10.24*	*6.09*	*− 2.06*	*85.0*	*86.0*
SII	1	a	27.32	62.92	39.59	22.13	26.49	17.29	6.31	+6.04	75.9	78.0
		b	28.47	62.79	38.82	22.09	23.89	20.33	6.57	+3.64	70.1	71.2
		average	*27.80*	*62.86*	*39.20*	*22.11*	*25.19*	*18.81*	*6.44*	*+4.84*	*73.0*	*74.6*
	2	a	26.71	63.50	36.59	22.28	21.82	20.21	6.17	+6.70	71.5	71.2
		b	27.21	63.41	34.85	22.28	21.12	18.83	6.29	+9.73	75.3	74.8
		average	*26.96*	*63.46*	*35.72*	*22.28*	*21.47*	*19.52*	*6.23*	*+8.22*	*73.4*	*73.0*
	3	a	27.12	61.93	41.35	21.87	25.72	13.10	6.26	+7.84	83.9	85.2
		b	27.90	62.10	43.18	21.92	27.24	14.11	6.44	+4.81	81.2	83.4
		average	*27.51*	*62.02*	*42.26*	*21.90*	*26.48*	*13.60*	*6.35*	*+6.14*	*82.6*	*84.3*
SIII	2	a	26.57	21.11	21.89	17.50	16.03	6.18	6.14	− 6.96	99.8	99.7
		b	26.23	21.11	22.22	17.50	16.38	6.40	6.06	− 7.51	97.9	97.8
		average	*26.40*	*21.11*	*22.06*	*17.50*	*16.20*	*6.29*	*6.10*	*− 7.24*	*98.8*	*98.8*
	3	a	28.20	21.11	22.10	17.50	15.61	7.47	6.51	− 8.46	94.2	93.4
		b	27.92	21.11	22.02	17.50	15.52	8.04	6.45	− 8.95	90.4	89.1
		average	*28.06*	*21.11*	*22.06*	*17.50*	*15.56*	*7.76*	*6.48*	*− 8.70*	*92.3*	*91.2*
SIV	2	a	27.07	51.38	33.24	21.16	23.52	9.54	6.25	+8.60	91.6	92.1
		b	27.54	51.38	33.42	21.16	21.79	9.51	6.36	+8.45	92.0	92.1
		average	*27.30*	*51.38*	*33.33*	*21.16*	*22.66*	*9.52*	*6.30*	*+8.52*	*91.8*	*92.1*
	3	a	28.16	51.38	32.52	21.16	24.18	9.84	6.50	+9.02	91.6	92.2
		b	28.48	51.38	32.39	21.16	20.81	10.24	6.58	+8.75	90.9	90.8
		average	*28.32*	*51.38*	*32.46*	*21.16*	*22.50*	*10.04*	*6.54*	*+8.88*	*91.2*	*91.5*
SV	4	a	36.01	18.06	19.64	18.42	14.14	7.92	8.32	− 9.50	102.4	103.2
		b	34.32	16.25	17.32	16.60	12.77	9.18	7.93	−10.25	92.0	89.3
		average	*35.16*	*17.16*	*18.48*	*17.51*	*13.46*	*8.55*	*8.12*	*− 9.88*	*97.2*	*96.2*
	5	a	36.06	17.11	24.61	16.76	19.79	10.59	8.33	−18.09	75.6	81.6
			36.82	17.07	23.73	18.18	18.17	7.92	8.51	−13.68	104.8	104.8

		average	*33.64*	*40.82*	*24.43*	*18.65*	*12.42*	*19.24*	*7.77*	*+3.13*	*72.0*	*67.0*
	5	a	33.59	46.82	21.85	18.65	10.25	24.14	7.76	+0.83	62.4	53.5
		b	33.74	46.82	24.19	18.65	11.46	24.18	7.79	- 1.55	60.3	51.9
		average	*33.66*	*46.82*	*23.02*	*18.65*	*10.86*	*24.16*	*7.78*	*- 0.36*	*61.4*	*52.7*
	6	a	33.79	46.82	23.43	18.65	12.43	19.02	7.81	+4.37	73.4	68.7
		b	34.14	46.82	21.05	18.65	11.45	19.66	7.89	+6.11	73.5	68.4
		average	*33.96*	*46.82*	*22.24*	*18.65*	*11.94*	*19.34*	*7.85*	*+5.24*	*73.4*	*68.6*
SVII	4	a	35.06	19.05	16.96	18.88	12.21	9.71	8.10	- 7.62	88.1	88.7
		b	35.28	19.05	15.95	18.88	10.87	9.54	8.15	- 6.44	93.7	90.0
		average	*35.17*	*19.05*	*16.46*	*18.88*	*11.54*	*9.62*	*8.12*	*- 7.03*	*90.9*	*89.4*
	5	a	35.47	19.05	19.25	18.88	13.19	8.45	8.19	- 8.65	98.6	98.0
		b	34.38	17.67	18.02	15.73	11.48	9.68	7.94	-10.03	88.7	84.4
		average	*34.92*	*18.36*	*18.64*	*17.30*	*12.34*	*9.06*	*8.06*	*- 9.34*	*93.6*	*91.2*
SVIII	4	a	39.82	60.36	28.94	19.98	16.44	28.19	9.20	+3.23	63.0	60.3
		b	40.18	60.55	24.46	20.14	15.94	27.10	9.28	+8.99	68.3	65.8
		average	*40.00*	*60.46*	*26.70*	*20.06*	*16.19*	*27.64*	*9.24*	*+6.11*	*65.6*	*63.0*
	5	a	39.17	60.15	28.71	19.77	13.95	31.22	9.05	+0.22	56.7	51.2
		b	38.72	60.55	29.49	20.14	16.37	31.51	8.94	- 0.45	55.9	52.4
		average	*38.94*	*60.35*	*29.10*	*19.96*	*15.16*	*31.36*	*9.00*	*- 0.12*	*56.3*	*51.8*
	6	a	40.42	60.01	31.92	19.81	15.91	27.08	9.34	+1.01	63.0	59.7
		b	39.74	60.55	31.40	20.14	16.57	25.68	9.18	+3.47	66.5	63.6
		average	*40.08*	*60.28*	*31.66*	*19.98*	*16.24*	*26.38*	*9.26*	*+2.24*	*64.8*	*61.6*
SIX	4	a	40.19	46.94	27.06	22.03	14.79	13.73	9.28	+6.15	89.4	87.2
		b	39.85	46.94	28.46	22.03	15.26	11.97	9.21	+6.51	93.2	91.8
		average	*40.02*	*46.94*	*27.76*	*22.03*	*15.02*	*12.85*	*9.24*	*+6.33*	*91.3*	*89.5*
	5	a	39.77	46.94	25.70	22.03	13.27	13.77	9.19	+7.47	89.4	86.7
		b	39.66	46.94	25.88	22.03	11.78	13.63	9.16	+7.43	89.6	86.4
		average	*39.72*	*46.94*	*25.79*	*22.03*	*12.52*	*13.70*	*9.18*	*+7.45*	*89.5*	*86.6*
	6	a	40.12	46.94	26.86	22.03	13.07	13.06	9.27	+7.02	91.0	88.6
		b	39.83	46.94	28.82	22.03	15.76	13.94	9.20	+4.18	88.2	86.0
		average	*39.98*	*46.94*	*27.84*	*22.03*	*14.42*	*13.50*	*9.24*	*+5.60*	*89.6*	*87.3*

Symbols and formulae of table 16

1. Metabolic faecal protein and absorbed protein are taken from table 15.
2. Endogenous protein (from literature) = 0.231 gm. per kg. of body weight.
3. Thomas–Mitchell formula for the calculation of the biological value is found in the text (chapter V).

3a. Metabolic faecal protein and endogenous 'protein' from the literature.

3b. Metabolic faecal protein = digestible crude protein was determined by the pepsin–HCl method, but endogenous protein taken from the literature.

TABLE 17. Biological values calculated by the different methods

Experiment	Lamb	Thomas-Mitchell method 3×		△-Methods				
		MFP calculated 3a×	*MFP* by pepsin-HCl 3b×	4	5	6	7	8
SI and SII	1	73.0	74.6	82.5	87.5	79.8	68.8	73.0
	2	73.4	73.0	58.4	73.4	51.2	44.5	55.9
	3	82.6	84.3	78.0	66.1	70.8	58.3	49.5
SIII and SIV	2	91.8	92.1	86.6	77.1	82.9	69.6	61.9
	3	91.2	91.5	90.6	80.1	88.5	74.7	65.6
Average of SV–SVII and SVI	4	72.0	67.0	52.3	53.6	53.2	52.2	53.3
	5	61.4	52.7	48.5	64.8	47.0	44.4	59.5
	6	73.4	68.6	61.1*	70.9*	62.1*	61.3*	71.1*

4. Biological value $= \frac{\triangle \text{absorbed protein} - \triangle(\text{urine protein} - \text{endogenous protein})}{\triangle \text{absorbed protein}} \times 100$
(metabolic faecal protein taken from literature).
($\triangle$ = results of moderate protein experiments minus results of their correspondings in protein-poor experiments, *e.g.*: results of SII minus results of SI).

5. Biological value = calculated by formula note 4, but metabolic faecal protein as determined by pepsin-HCl method.

6. Biological value $= \frac{\triangle \text{protein balance}}{\triangle \text{digested protein}} \times 100$ (for $\triangle$ see note 4.).

7. Biological value $= \frac{\triangle \text{protein balance}}{\triangle \text{absorbed protein}} \times 100$ (for $\triangle$ see note 4.; metabolic faecal protein taken from the literature).

8. Biological value is calculated by formula note 7., but metabolic faecal protein as determined by pepsin-HCl method.

× for symbols and formulae see foot note at the end of table 16.

* SV and SVI.

TABLE 18. Average digestion coefficients of the dry matter

Experiment	Protein-poor ration	Experiment	Moderate-protein ration
SI	69.7	SII	72.3
SIII	60.1	SIV	68.6
SV, SVII	72.2	SVI	79.6

TABLE 19. Digestibility of proteins in the rations secured experimentally and computed with the formulae b and c of HUISMAN (1946)

Experiment	Formula		Secured experimentally	Experiment	Formula		Secured experimentally
	b	c			b	c	
SI	3.68	3.61	1.56	SV	–0.14	–0.60	–0.83
SII	5.80	5.95	4.03	SVI	4.72	4.76	4.73
SIII	0.57	0.18	–0.21	SVII	0.00	–0.45	0.22
SIV	4.50	4.51	3.28	SVIII	6.30	6.50	5.82
				SIX	3.51	3.42	3.35

Protein balances (tables 16 and 20) – As might be expected, negative balances occurred with the protein-poor rations in SI, SIII, SV and SVII. With the rations of higher protein content the balances were positive with some few exceptions. These refer to lamb no. 5, which showed balances just below equilibrium in SVI and SVIII, whilst, in the same experiments, the balances of the other two lambs (nos. 4 and 6) were definitely positive. We noticed that in SV and SVII, on low protein diets, the balances of lamb no. 5 were also lower than those of nos. 4 and 6. On the contrary, in SIX (more protein) the balances of lamb no. 5 were somewhat higher than those of nos. 4 and 6.

In experiments with rats and pigs it is customary to define the protein levels of the diets as percentages of crude protein in the dry matter. This is a good criterion because the feeds of these animals are highly digestible. With roughages however, such as hay and straw, utilized in ruminant nutrition, the *digestibility* should also be taken into account. Moreover, it should be considered that in rations with moderate and low protein contents the *apparent* digestibility is not a definite value, but that it is to a high degree dependent on the protein content in the dry matter. Presumably, this is the most important reason why in our experiments the quantity of digested protein (protein intake minus faecal protein) is considerably lower than that calculated with the aid of digestion coefficients from the literature. For all these reasons we have chosen absorbed crude protein (protein intake minus faecal protein plus calculated metabolic faecal protein) for characterizing the protein levels (table 20).

There proved to be a very significant positive regression of the protein balance on absorbed crude protein level as may be seen from fig. 1. This however does not alter the fact that the regression of the protein balance on calculated digestible crude protein was also distinctly positive. A zero balance was

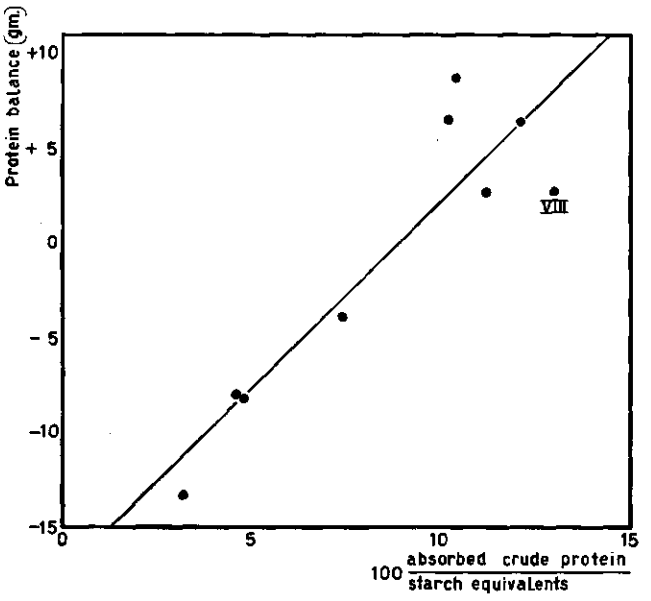

FIG. 1. Correlation between protein balance and absorbed protein per 100 gms. of starch equivalents. (VIII = value obtained in experiment SVIII).

obtained at $\pm 9\%$ absorbed crude protein per 100 starch units (100 gms. *SE*) and at $\pm 6\%$ calculated digestible crude protein per 100 starch units.

In fig. 1 the deviation from the regression line is greatest for experiment SVIII (autumn hay). The protein balance on this hay was much lower than expected from the regression line. Below, it will be seen that this involves also a low biological value.

Biological values (tables 16, 17 and 20) – These were calculated with the aid of the following formulae:

(1) THOMAS-MITCHELL's formula:

$$BV = \frac{I - (FP - MFP) - (UP - EP)}{I - (FP - MFP)} \times 100,$$

in which BV = biological value, I = protein intake, FP = faecal protein, MFP = metabolic faecal protein, UP = urinary protein and EP = endogenous protein. Figures for MFP and EP were taken from the literature.

(2) The same THOMAS-MITCHELL formula; this time MFP was taken from the pepsin-HCl determinations in the faeces.

As there were large differences between the MFP-values calculated and those determined (tables 15 and 16), one might expect large differences in the BV calculated after formulae (1) and (2). This, however, turned out not to be so, because THOMAS-MITCHELL's formula is not very sensitive to changes in MFP; the difference between the MFP calculated and determined is namely small as compared with the total protein intake (I).

This may be seen from table 20, where the figures referring to the experiments with moderate protein are in italics.

TABLE 20. Some average data

Hay tested in the different experiments	Crude protein in dry matter (%)	Digestible crude protein calculated		Absorbed crude protein per 100 gms. *SE*	Protein balances	Biological values		
		in dry matter (%)	per 100 gms. *SE*			calculated by formula 1	calculated by formula 2	Average
Early spring hay (1958), SI	7.6	3.8	5.8	7.4	– 3.9	77.6	80.6	79.1
„ „ „ (1958), SII	*9.8*	*5.9*	*10.0*	*12.1*	*+6.4*	*76.3*	*77.3*	*76.8*
„ „ „ (1958), SIII	4.2	2.5	3.4	4.6	– 8.0	95.6	95.0	95.3
„ „ „ (1958), SIV	*8.4*	*4.8*	*7.8*	*10.4*	*+8.7*	*91.5*	*91.8*	*91.6*
„ „ „ (1959), SV	3.2	2.1	2.5	3.2	–13.3	91.9	93.7	92.8
„ „ „ (1959), SVI	*8.6*	*4.9*	*7.1*	*11.2*	*+2.7*	*68.9*	*62.8*	*65.8*
„ „ „ (1959), SVII	3.5	2.0	2.6	4.8	– 8.2	92.2	90.3	91.2
Autumn „ (1959), SVIII	*10.3*	*6.1*	*9.0*	*13.0*	*+2.7*	*62.2*	*58.8*	*60.5*
Late spring „ (1959), SIX	*7.3*	*3.9*	*6.1*	*10.2*	*+6.5*	*90.1*	*87.8*	*89.0*
Moderate protein experiments (av. of SII, SIV, SVI, SVIII and SIX)	*8.9*	*5.1*	*8.0*	*11.4*	*+5.4*	*77.8*	*75.7*	*76.7*
Low protein experiments (av. of SI, SIII, SV and SVII)	4.6	2.6	3.6	5.0	– 8.4	89.3	89.9	89.6
Spring hay experiments (av. of SII, SIV, SVI and SIX)	*8.5*	*4.9*	*7.8*	*11.0*	*+6.1*	*81.7*	*79.9*	*80.8*

In the moderate protein experiments the average *BV* of all the hays is 77.8 when calculated by formula 1, while it is 75.7 when calculated by formula 2. The close agreement between these formulae is also found in the low protein experiments, the averages being 89.3 and 89.9 respectively. From these figures we may also conclude that the absorbed protein was more efficiently utilized in the low protein rations than in the rations with more protein.

As already mentioned in chapter VII, several Δ-formulae (nos. 4, 5, 6, 7, 8) were also tried, where Δ means: result of moderate protein experiment (*e.g.* result of SII) minus result of low protein experiment with the same kind of hay (*e.g.* result of SI).

The results secured with these formulae are shown in table 17. It is obvious that they are very variable, ranging from 44.4 to 90.6. Furthermore, they are in general distinctly lower than those of the THOMAS-MITCHELL formula. One of the reasons for these divergencies is probably that the accuracy of the Δ-formulae is impaired by the fact that the results are calculated as differences so that the errors accumulate. That the results are lower than those of formulae 1 and 2 may be caused by the more efficient utilization of the protein in the low protein diets as compared with the corresponding moderate protein rations. It seemed therefore the best plan to drop the results of the Δ-formulae and to take into account only the results of the THOMAS-MITCHELL formulae 1 and 2.

Although not as large as the above mentioned variation, the range of the results of these formulae 1 and 2 was also considerable, not only between animals (table 16) on the same hay, but also between experiments with the same animal and the same kind of hay (tables 16 and 20), as may be seen from the differences between SII and SIV. It has been shown several times in the literature that biological values decrease with higher protein levels and conversely. This explains partly the higher *BV* of SIV as compared with SII on the same hay. It remains however obscure why the *BV* in SI with a protein level of 5.8% is lower than in SIV with a protein level of 7.8%.

The *BV* secured with the autumn hay (SVIII; average 60.5) is lower than any of those obtained with the three spring hays (average 80.8). From table 12 it may be seen that the autumn hay contains the highest percentage of protein, namely 20% crude protein in the dry matter. The amounts of absorbed and digestible crude protein per 100 gms. *SE* ingested in SVIII are however about the same as in SII (*BV* 77, table 20), so that the high protein content of the autumn hay cannot be the only reason for the low *BV* found in SVIII.

As far as grass is concerned, SYNGE (1952) mentioned that the proportions of amino acids in the true protein of the leaf vary little with season and species. Furthermore he emphasized that the value of protein and non-protein N to a ruminant animal is not only determined by the amino acid composition but also by the amount and nature of the carbohydrate that is being simultaneously fermented in the rumen, and also by the solubility of the protein and its susceptibility to the microbial proteases (see also chapter II). In this connection he reminds of the fact that the content of soluble carbohydrate in grass fluctuates greatly with the season. It seems possible to him that these changes rather than changes in the protein content may be responsible for some of the differences that are said to exist between the value of spring and those of autumn grass.

In our experiments, however, the low *BV* cannot be explained by a low content of soluble carbohydrates in the autumn grass, because the amount of

maize starch in the whole diet was greater than in any of the other experiments. As mentioned before we also consider it improbable that the low *BV* was caused by the fact that the provision with starch equivalents in this experiment was lower than in the other experiments. It might however be possible that the amount of easily fermentable non-protein-nitrogen-compounds has been excessively high or that the heating in the hay stack may have affected the protein in the SVIII-hay more intensively than in the other hays. It seems to us that the influence of the heating in the hay stack on the solubility, on the susceptibility to rumen fermentation and on the *BV* of hay proteins should be studied more closely in the near future.

From the above mentioned discussion we see that the three grass hays cultivated in spring had an average biological value of 81; for the autumn hay it was 60. The values secured in our investigation are rather high and indicate a much higher percentage utilization of protein than has been reported by other investigators. For instance, the value of spring hay is much higher than those reported by SOTOLA (1930 and 1933) with a crude protein level of about 14% in the dry matter, SMUTS *et al.* (1938) with 8% and MILLER *et al.* (1939) with 10%, in their experiments with sheep fed on lucerne hay. The biological values secured in their investigations were 56, 51, 60 and 59 respectively. In our experiments the digestible crude protein level calculated per 100 gms. *SE* was 7.8 for the rations with the spring hays or 8.5% crude protein in the dry matter. This difference in protein level is not so large as to account for the differences between our figures and those of the above mentioned authors.

TURK *et al.* (1934) obtained higher biological values for the lucerne and clover proteins, the figures being 79 and 81 respectively. These values are quite similar to those of our spring hays (81). Considering the wide variations between their results and those of SOTOLA, TURK *et al.* conducted an experiment in which lambs were fed with lucerne hay alone as was done in SOTOLA's experiments. In a second trial the lucerne hay was supplemented with starch, sugar, cellulose, maize oil, and minerals. The protein level in the first experiment was 18.6% in the dry matter, while in the second experiment it was about 10.5%. The contents of crude fibre and the amounts of dry matter eaten were about the same. Biological values of 50 and 72 were obtained in the first and second experiments respectively. The latter figure agreed fairly well with the value of 77 secured with the same lambs in another experiment preceding these two trials, which was performed for determining the *BV* of lucerne hay protein in a ration just the same as the supplemented one. From this they concluded that the low biological values obtained for the lucerne protein when fed alone to sheep might be due not to any deficiency of the protein but to the method of experimentation, for instance the protein level or the net energy supply.

Moreover, the wide difference in plane of protein intake may have been a chief factor in causing the great difference that had been observed by SOTOLA (1930) when estimating the biological values of the protein in maize silage (94) and in lucerne hay (56) as each was fed as a single feed to sheep. The same reason may be valid in explaining the difference found in biological values secured by the same author in 1933 when determining the *BV* of protein in lucerne stems (containing about 9% protein on dry matter basis) and lucerne leaves (containing about 21% protein); the figures reported were 64 and 44 respectively.

In concluding this discussion, we may say that spring hays as examined in our

experiments had biological values which exceeded most of those reported in the literature for the lucerne hay, with the exception of those of TURK *et al.* (1934) which showed a very close agreement with our results. Some of the reasons for the high values in our experiments with spring hays may be due to the low level of protein fed to the animals and the liberal supply of energy.

PART III. CONCLUSIONS

SUMMARY AND CONCLUSIONS

Nine metabolism experiments were conducted with six male lambs of the Texel breed in order to determine the biological value of proteins in grass hays from ordinary pastures in the surroundings of Wageningen (The Netherlands). This study was carried out during the years 1959 and 1960. Four kinds of hays were examined: early spring hay 1958 (SI–SIV); early spring hay 1959 (SV–SVII); autumn hay 1959 (SVIII) and late spring hay 1959 (SIX).

In the first part of this publication (ch.I – ch.VI) the literature was reviewed. The second part describes the materials and methods that were used in this investigation together with the discussion of the results secured. In the third part we give a brief summary and draw our conclusions.

INTRODUCTION

Proteins are indispensable for supporting animal life. Their quality is an important factor in determining the quantity of protein needed for the various physiological functions. For most animals difference in protein quality depends mainly upon digestibility and amino acid composition. Nevertheless it is difficult to predict the nutritive value of a given protein from its amino acid composition; therefore biological examinations should be carried out. This applies especially to the ruminant animal, because degradation and synthesis of amino acids may be effected by the rumen microorganisms.

CHAPTER I. ESSENTIAL AND NONESSENTIAL OR INDISPENSABLE AND DISPENSABLE AMINO ACIDS

From 8 to 10 amino acids have proved to be indispensable for the growth and the maintenance of mammalian body tissues. In the text it is shown that the various species essentially require the same pattern of amino acids, men and honey-bees included. Undoubtedly the ruminant tissues also need the same amino acid pattern. It is however not necessary that all these acids should be present in their food on account of the synthetic activities of the rumen micro-organisms.

CHAPTER II. DEGRADATION AND SYNTHESIS OF PROTEINS AND AMINO ACIDS BY RUMEN MICROORGANISMS

A variable quantity of protein is broken down in the fore-stomachs. The end products of this degradation are mainly ammonia, CO_2 and volatile fatty acids. It has been shown that besides breakdown there is also synthesis of protein by the rumen organisms, even when urea is the only source of N. The synthe-

sized protein proved to be of high nutritive value and to contain a satisfactory amino acid pattern.

CHAPTER III. METABOLIC FAECAL NITROGEN (PROTEIN) (*MFN* AND *MFP*)

The metabolic faecal nitrogen is the amount of nitrogen in the faeces originating from intestinal secretions. The figure of *MFN* (*MFP*) is an essential element in the calculation of the biological value (*BV*) by the THOMAS-MITCHELL formula. Yet it has been shown that the figure for *BV* is not very sensitive to inaccuracies in the estimation of *MFN*. The direct and indirect methods for determining the *MFN* and the factors affecting its amount have been reviewed.

A comparison has been made between the figures for *MFN* determined with various methods. We have chosen the figure of 0.55 gm. N (or 3.438 gm. protein) per 100 gms. ingested dry matter for computing the *BV* in our experiments. This figure was preferred because it has been obtained experimentally with sheep fed on N-free rations. In calculating the *BV* we have also used the figures for *MFN* which were obtained from our pepsin-HCl determinations.

CHAPTER IV. ENDOGENOUS NITROGEN (PROTEIN) (*EN* AND *EP*)

A distinction between two kinds of protein catabolism, endogenous and exogenous, was first suggested by FOLIN in 1905. The endogenous protein catabolism is demonstrated by the constant excretion of creatinine and other compounds in the urine. The more variable constituents of urine, mainly urea, are considered to be for a large part the end products of the exogenous protein catabolism. This theory of FOLIN is discussed in the text. The *EN*, just as the *MFN*, can be determined when feeding the animals on N-free rations. Only few results of such experiments with sheep on N-free rations have been found in the literature. The average figure for *EN* was 0.037 gm. of N (or 0.231 gm. protein) per kg. of body weight. This figure was used by us in calculating the *BV* with the aid of the THOMAS-MITCHELL formula of which the *EN* is an essential part.

CHAPTER V. METHODS FOR DETERMINING THE BIOLOGICAL VALUE OF PROTEINS

The methods for determining the biological value (*BV*) of proteins are reviewed. In addition, the factors affecting the *BV* are also discussed. The methods of THOMAS-MITCHELL and the method of the nitrogen balance index were chosen as being the most suitable for determining the biological value of proteins in hays with the aid of ruminants.

CHAPTER VI. BIOLOGICAL VALUE OF PROTEINS IN ROUGHAGES

Few experiments have been conducted to determine the *BV* of the proteins in common roughages. Some authors suggested that, in ruminants, all kinds of protein fed at a level of 10 to 12% on dry matter basis would have biological values of approximately 60. Later on, however, other research workers obtained higher biological values with sheep. The large variation of the results secured might be due to differences in protein concentration, energy intake, dry matter intake and technique of experimentation. For a good comparison such factors should be standardized as far as possible.

CHAPTER VII. MATERIALS AND METHODS

The materials and methods used in this investigation and also the experimental animals, the diets and the collection of samples are described in detail. The scheme of our study was to give the experimental lambs low protein rations (SI, SIII, SV and SVII) successively followed by moderate protein ones (SII, SIV and SVI) leaving no feed residues. SVIII and SIX were mainly designed to compare the *BV* of autumn hay and late spring hay harvested in the same year (1959). All the rations were composed in such a way as to provide mainly the net energy required for the maintenance only.

CHAPTER VIII. RESULTS AND DISCUSSION

Diets (table 11): The diets consisted of hay, potato or maize starch, cellulose, carrots meal and minerals.

The protein-poor diets contained 3.2 to 7.6% of crude protein per 100 gms. of dry matter. In these diets the hay protein ranged from 67.9 to 71.8% of the total amount of protein. The amount of digestible crude protein was 2.0 to 3.8% per 100 gms. of dry matter and 2.5 to 5.8 gm. per 100 gms. of starch value (*SE*).

In the diets with moderate protein content the percentage of crude protein amounted to 7.3 to 10.3% on dry matter basis. The hay protein varied from 87.0 to 92.4% of the total amount of protein. These diets contained 3.9 to 6.1 gms. of digestible crude protein per 100 gms. of dry matter and 6.1 to 10.0 gms. per 100 gms. of starch value.

Digestibility (table 15): The average digestibility of the dry matter in the experimental rations (moderate protein experiments) was compared with that of the corresponding control rations (protein-poor experiments). The ranges were from 60 to 80% in all the experiments. The apparent digestibility of the crude protein was variable being lowest in the protein-poor control diets (−17.2 to +22.8%) and much higher in the experimental ones (+31.8 to 55.8%). A large part of this variation is caused by differences in the protein contents in the diets. When the apparent digestibility was corrected for *MFP* with figures from the literature the majority of the true protein digestion coefficients ranged from 61.1 to 94.4%. When the digestibility was corrected with figures as determined with pepsin-HCl the range was from 65.6 to 82.6%.

Protein balances (table 16): With protein-poor rations we obtained negative protein balances ranging from −3.9 to −13.3 gms. of protein per day. Positive balances from +2.7 to +8.7 gms. of protein were secured on diets with higher protein contents.

Biological values (*BV*) (table 16): The THOMAS-MITCHELL formula was used with modifications. In the first modification the figure for *MFP* was taken from the literature (3.438 gm. protein per 100 gms. of ingested dry matter). In the second modification the same formula was used but with the figure for *MFP* obtained from pepsin-HCl determinations in the faeces. The biological values obtained by the two procedures were in good agreement, both in the low protein experiments and the higher protein ones. The average *BV* of all hays in the low protein control experiments was 89.3 and 89.9 as calculated with modification 1 and 2 respectively. For diets with moderate protein content the values were successively 77.8 and 75.7.

Employing several modifications of the nitrogen balance index method the results were very variable ranging from 44.4 to 90.6 (table 17). As a rule they were distinctly lower than those obtained by the THOMAS-MITCHELL method.

The *BV* secured with the autumn hay (60.5; SVIII) was lower than any of those obtained with the three spring hays (average 80.8). The crude protein level in the dry matter and the amount of digestible crude protein per 100 gms. of dry matter and per 100 gms. of starch value were somewhat lower in the spring hays than in the autumn hay (table 20). This difference in protein levels may be one of the reasons why the *BV* in the autumn hay was lower. Moreover, it might be possible that in the autumn hay the amount of easily fermentable non-protein-nitrogen-compounds has been excessively high or that the heating in the hay stack may have affected the protein in SVIII more intensively than in the other hays. Nevertheless, it may be stated that the *BV* of the proteins in the spring hays was much higher than those reported in the literature for lucerne or clover hay proteins, with the exception of the results obtained by TURK *et al.* (1934) which were very similar to ours.

SAMENVATTING EN CONCLUSIES

Om de biologische waarde te bepalen van de eiwitten in grashooi van gewone weiden rondom Wageningen, werden negen verteringsproeven uitgevoerd met zes ram lammeren van het Texelse ras. Deze proeven werden genomen in de jaren 1959 en 1960. Er werden vier soorten hooi onderzocht, nl.: vroeg voorjaarshooi 1958 (proef SI–SIV), vroeg voorjaarshooi 1959 (SV–SVII), najaarshooi 1959 (SVIII) en laat voorjaarshooi 1959 (SIX). In het eerste deel van dit werk (hoofdstukken I–IV) wordt de literatuur op dit gebied behandeld. Het tweede deel (hoofdstukken V–VIII) beschrijft het materiaal en de methoden, welke bij dit onderzoek werden gebruikt. Ook worden hierin de verkregen uitkomsten besproken. In het derde deel worden na een korte samenvatting onze conclusies opgesomd.

INLEIDING

Eiwitten zijn noodzakelijk om het dierlijk leven in stand te houden. De kwaliteit ervan is van groot belang voor de bepaling van de hoeveelheid, die nodig is voor de verschillende fysiologische functies. Voor de meeste dieren wordt de kwaliteit van het eiwit hoofdzakelijk bepaald door de verteerbaarheid ervan en door de aminozuren, waaruit het is opgebouwd. Toch is het niet eenvoudig om, uitgaande van de aminozuur-samenstelling, de voedingswaarde van een bepaald eiwit te voorspellen. Hiervoor zouden biologische bepalingen moeten worden uitgevoerd. Dit is vooral het geval voor herkauwers, omdat in de pens afbraak en opbouw van aminozuren kan plaats hebben onder invloed van micro-organismen.

HOOFDSTUK I. NOODZAKELIJKE EN NIET NOODZAKELIJKE AMINOZUREN

Van ongeveer 8 tot 10 aminozuren is vastgesteld, dat zij noodzakelijk zijn voor de groei en de instandhouding van de weefsels van zoogdieren. In de tekst wordt er op gewezen, dat de verschillende diersoorten, mensen en bijen niet uitgezonderd, eenzelfde aminozuurpatroon nodig hebben. Ongetwijfeld behoeven

ook de weefsels van de herkauwers hetzelfde aminozuurpatroon. Toch is het vanwege de synthetische activiteiten van de pensbacteriën niet noodzakelijk, dat al deze aminozuren in het voedsel gepreformeerd aanwezig zijn.

HOOFDSTUK II. AFBRAAK EN OPBOUW VAN EIWITTEN EN AMINOZUREN DOOR PENSBACTERIËN

Een wisselende hoeveelheid eiwit wordt in de voormagen afgebroken. De eindprodukten van deze afbraak zijn voornamelijk: ammonia, CO_2 en vluchtige vetzuren. Er is aangetoond, dat naast afbraak ook opbouw van eiwitten door pensbacteriën plaats heeft, ook zelfs wanneer ureum de enige stikstofbron is. Het opgebouwde eiwit bleek een hoge voedingswaarde te hebben en een goed aminozurenpatroon.

HOOFDSTUK III. METABOLISCH FAECAALSTIKSTOF (-EIWIT) (*MFN* EN *MFP*)

Metabolisch faecaalstikstof is die hoeveelheid stikstof in de faeces die van de secretie der spijsverteringsorganen afkomstig is. De waarde van het metabolisch faecaaleiwit is van groot belang voor de berekening van de biologische waarde (*BV*) met de formule van THOMAS en MITCHELL. Toch is er aangetoond, dat het getal voor de biologische waarde niet erg gevoelig is voor onnauwkeurigheden in de bepaling van de metabolische faecaalstikstof. Directe en indirecte methoden om het metabolisch faecaaleiwit te bepalen worden besproken. Er werd een vergelijking gemaakt tussen de waarden voor metabolisch faecaalstikstof, die volgens verschillende methoden werden bepaald. Om de biologische waarde voor onze proeven te berekenen, hebben wij een waarde van 0,55 g N (of 3,438 g eiwit) per 100 g opgenomen droge stof gekozen. Wij hebben aan deze waarde de voorkeur gegeven, omdat deze werd verkregen uit proeven met schapen op stikstofvrije rantsoenen. Daarnaast hebben wij voor de berekening van de biologische waarde de uitkomsten gebruikt, die wij met onze eigen pepsine-zoutzuur-bepalingen hadden verkregen.

HOOFDSTUK IV. ENDOGENE STIKSTOF (EIWIT) (*EN* EN *EP*)

In 1905 werd voor het eerst door FOLIN voorgesteld een onderscheid te maken tussen twee soorten van eiwitstofwisseling, nl. de endogene en de exogene. De endogene eiwitstofwisseling werd aannemelijk gemaakt door de constante uitscheiding van creatinine en andere stoffen in de urine. De afbraakprodukten die in wisselende hoeveelheden in de urine voorkomen, voornamelijk ureum, werden beschouwd als voor het merendeel afkomstig te zijn van de exogene eiwitstofwisseling. Deze theorie van FOLIN wordt besproken. De endogene stikstof kan, evenals de metabolische faecaalstikstof, bepaald worden met dieren op stikstofvrije rantsoenen. In de literatuur worden slechts enkele van zulke proeven met schapen op stikstofvrije rantsoenen vermeld. De gemiddelde waarde, die voor de endogene stikstof werd gevonden, bedraagt 0,037 g N (of 0,231 g eiwit) per kg lichaamsgewicht. Deze waarde werd door ons gebruikt bij de berekening van de biologische waarde met behulp van de formule van THOMAS en MITCHELL. Endogene stikstof is hierin een belangrijke factor.

HOOFDSTUK V. METHODEN VOOR DE BEPALING VAN DE BIOLOGISCHE WAARDE VAN EIWIT

De bestaande methoden voor de bepaling der biologische waarde (*BV*) van eiwitten werden behandeld. Bovendien werden de factoren besproken, die van invloed zijn op de biologische waarde. De methode van THOMAS-MITCHELL en de methode van de stikstofbalans-index werden uitgekozen als zijnde de meest geschikte om de biologische waarde van eiwitten in hooi voor herkauwers te bepalen.

HOOFDSTUK VI. DE BIOLOGISCHE WAARDE VAN EIWIT IN RUWVOEDERS

Er zijn slechts weinig proeven verricht om de biologische waarde van eiwitten in de gebruikelijke ruwvoeders te bepalen. Sommige auteurs meenden, dat voor herkauwers alle soorten eiwit een biologische waarde van ± 60 zouden hebben, indien zij gevoerd worden in gehalten van 10 tot 12%, berekend op de droge stof. Later werden echter door andere onderzoekers hogere waarden gevonden met schapen. De grote verschillen in de verkregen waarden zouden veroorzaakt kunnen worden door verschillen in de eiwitgehalten, of in de energie opname, of in de hoeveelheden opgenomen droge stof, dan wel in de uitvoering van de bepaling. Voor een goede vergelijking zouden al deze factoren dus zo goed mogelijk constant moeten worden gehouden.

HOOFDSTUK VII. MATERIAAL EN METHODEN

De materialen en de methoden, die bij dit onderzoek werden gebruikt, alsook de proefdieren, de samenstelling van het voer en het verzamelen van de monsters worden uitvoerig besproken. De proefopzet was om de lammeren rantsoenen met een laag eiwitgehalte te voeren (SI, SIII, SV en SVII), gevolgd door rantsoenen met een hoger eiwitgehalte (SII, SIV en SVI), terwijl er voor gezorgd werd, dat er weinig of geen voedselresten waren. SVIII en SIX dienden hoofdzakelijk om de biologische waarde van voorjaars- en najaarshooi, in eenzelfde jaar geoogst, te vergelijken. De rantsoenen waren zodanig samengesteld, dat vrijwel alleen de hoeveelheid netto-energie werd gegeven, die voor het onderhoud nodig was.

HOOFDSTUK VIII. UITKOMSTEN EN DISCUSSIE

Rantsoenen (tabel 11): De rantsoenen waren samengesteld uit hooi, aardappel- of maiszetmeel, cellulose, rode wortelen-meel en mineralen.

De eiwitarme rantsoenen bevatten 3,2 tot 7,6% ruweiwit per 100 g droge stof. In deze rantsoenen vertegenwoordigde het hooi-eiwit 67,9 tot 71,8% van het totale eiwitgehalte. Het gehalte van verteerbaar ruweiwit was 2,0 tot 3,8 g per 100 g droge stof of 2,5 tot 5,8 g per 100 g zetmeelwaarde (*SE*).

De rantsoenen met een hoger eiwitgehalte bevatten van 7,3 tot 10,3% ruweiwit, berekend op de droge stof. Het hooi eiwit varieerde van 87,0 tot 92,4% van het totaal-eiwit. Deze rantsoenen bevatten 3,9 tot 6,1 g verteerbaar ruweiwit op 100 g droge stof of 6,1 tot 10,0 g per 100 g zetmeelwaarde.

Verteerbaarheid (tabel 15): De gemiddelde verteerbaarheid van de droge stof in de proefrantsoenen (proeven met een hoger eiwitgehalte) werd vergeleken met die van de controle-rantsoenen (eiwit-arme rantsoenen). De waarden van alle

proeven lagen tussen 60 en 80%. De schijnbare verteerbaarheid van het ruw-eiwit was zeer verschillend; het laagst lagen de eiwitarme controle-rantsoenen (−17,2 tot +22,8%) en veel hoger voor de proefrantsoenen (+31,8 tot 55,8%). Voor een groot gedeelte wordt deze schommeling veroorzaakt door de verschillen in eiwitgehalte van de rantsoenen. Wanneer de schijnbare verteerbaarheid gecorrigeerd werd voor metabolisch faecaaleiwit met behulp van cijfers uit de literatuur, kwam het merendeel van de verteringscoefficiënten van het eiwit tussen 61,1 en 94,4% te liggen. Wanneer de schijnbare verteerbaarheid werd gecorrigeerd met de waarden, die bij de pepsine-zoutzuur-bepalingen waren verkregen, dan werden verteringscoëfficiënten tussen 65,6 en 82,6 gevonden.

Eiwitbalansen (tabel 16): Met de eiwitarme rantsoenen verkregen wij negatieve balansen van −3,9 tot −13,3 g eiwit per dag. Een positieve balans van +2,7 tot +8,7 g eiwit per dag werd verkregen met de rantsoenen, die méér eiwit bevatten.

Biologische waarde (*BV*) (tabel 16): De formule van THOMAS-MITCHELL werd in enige variaties toegepast. Voor de eerste toepassing werd een waarde voor het metabolisch faecaaleiwit genomen uit de literatuur (3,438 g eiwit per 100 g opgenomen droge stof). Voor de tweede toepassing werd dezelfde formule genomen; maar nu werd voor het metabolisch faecaaleiwit de waarden gebruikt, die met de pepsine-zoutzuur-bepalingen waren verkregen. De biologische waarden, die met beide toepassingen werden gevonden, stemden goed overeen, zowel voor de eiwitarme proeven als voor die met meer eiwit.

De gemiddelde biologische waarden voor alle hooisoorten was bij de proeven met eiwitarme rantsoenen 89,3 en 89,9, respectievelijk uitgerekend met formule 1 en 2. Voor de rantsoenen met meer eiwit waren deze waarden 77,8 en 75,7.

Wanneer de stikstofbalans-index-methode werd gebruikt in verschillende modificaties, liepen de waarden zeer uiteen, nl. van 44,4 tot 90,6. Als regel waren ze aanmerkelijk lager dan de waarden die met de THOMAS-MITCHELL methode werden gevonden.

De biologische waarde die voor het najaarshooi werd gevonden (60,5; SVIII), was lager dan de waarden die met de verschillende soorten voorjaarshooi werden verkregen (gemiddeld 80,8). Het is echter zeer goed mogelijk, dat in het najaarshooi een bijzonder hoog gehalte aan gemakkelijk te fermenteren amiden voorkwam of dat hooibroei het eiwit in SVIII meer had aangetast dan dat in de andere hooisoorten. Evenwel kan worden vastgesteld, dat de biologische waarden van de eiwitten in het voorjaarshooi veel hoger waren dan die, welke in de literatuur vermeld worden voor de eiwitten in lucerne en klaver hooi met uitzondering van de waarden door TURK *et al.* (1934) gevonden, welke met de onze overeenstemden.

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