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The Endogenous Nitrogen Metabolism of Sheep with Special Reference to the Maintenance Requirement of Protein.

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SINCE the classic work of Folin in 1905, in which he differentiated between an exogenous and an endogenous form of protein catabolism, much work has appeared to test the validity of his postulation. While there exists a general agreement on the exogenous form of protein catabolism, the literature is somewhat divided on the nature and biological importance of the endogenous form of protein metabolism. Folin regarded the latter form of catabolism as a constant type of catabolism in comparison with the more variable excretion of nitrogenous end-products arising from exogenous origin. Osborne and Mendel (1914) as well as Sherman (1920) question the constancy of the endogenous nitrogen and claim that if a completely efficient protein is administered, the endogenous nitrogen would be minimized. Mitchell (1930), however, who investigated this point very carefully by supplying small quantities of protein of a high biological value, could not find any evidence in support of the theory of the above workers, but agrees with Folin, that the endogenous nitrogen is a biological constant. Smuts (1935) found a definite relationship between the endogenous nitrogen metabolism and the basal metabolism of different species of animals varying widely in weight. Since the latter measurement is fully recognized as being reproducible and constant for an individual, it follows that the endogenous nitrogen is a measure of equal constancy.

Furthermore the preponderance of evidence in the literature definitely favours a constant output of endogenous nitrogen, which can be related either to body weight or surface area.

While extensive work has been conducted on the endogenous nitrogen metabolism of rats and other types of omnivora, scanty attention has been given to this type of metabolism in ruminants.

This fact is mainly accounted for by the extreme difficulties encountered in the computation of suitable nitrogen-free rations, which are palatable and easily consumed. Nevertheless Morgin (1912) and his co-workers, Satola (1930), and Turk and his collaborators (1934), succeeded to induce sheep to eat a low nitrogen ration consisting mainly of straw, starch, sugar, oil and minerals. In our study on the determination of the biological values of feeds for larger farm animals, it became essential to compound a suitable nitrogen-free ration for sheep. This has been successfully executed and the results reported in this text.

EXPERIMENTAL.

Mature merino wethers were used throughout this study. The best eaters were selected and were then put on an exclusive lucerne ration for a month. After this, they were directly transferred to the metabolism cages of the Forbes type and put on a nitrogen-free ration. Quantitative collection of the urine in 2 per cent. HCl was carried out daily and analysed for total nitrogen (Kjeldahl method).

Creatinine (creatinine and creatine), total sulphur, and neutral sulphur were determined. The creatinine was determined according to the microchemical modification of Folin's method as reported by Folin (1924). The total sulphur was determined by method of Denis (1910). Gas flames were avoided in all evaporating processes. The faeces were collected daily, stored in airtight jars, and aliquots analysed for total Nitrogen at the end of the collection period. Sheep were fed once daily.

The composition of the nitrogen free ration is given in Table 1. The final composition as reported is the outcome of several attempts in which certain ingredients were omitted and others added. It was found that the inclusion of sugar, even at as low a level as 5 per cent., invariably caused looseness of the bowels. The addition of different types of oils was tested out. It was found that they either did not mix well with the ration or put the sheep completely off feed. Ordinary maize starch was found to be too pulverized with the result that dextrinized starch was resorted to. This proved very successful. The dextrinized starch was ground into small pieces equivalent in size to the maize kernel. These the sheep consumed excellently and some even preferred it to maize. When putting sheep on the nitrogen-free ration, it was found advisable to start them off with 150 to 200 grams of the mixture and increase the daily feed until they just cleared up their daily allotment. With this ration under our conditions, we did not experience any difficulty with feed refusal or reduction in weight.

TABLE I.

Composition of Ration.

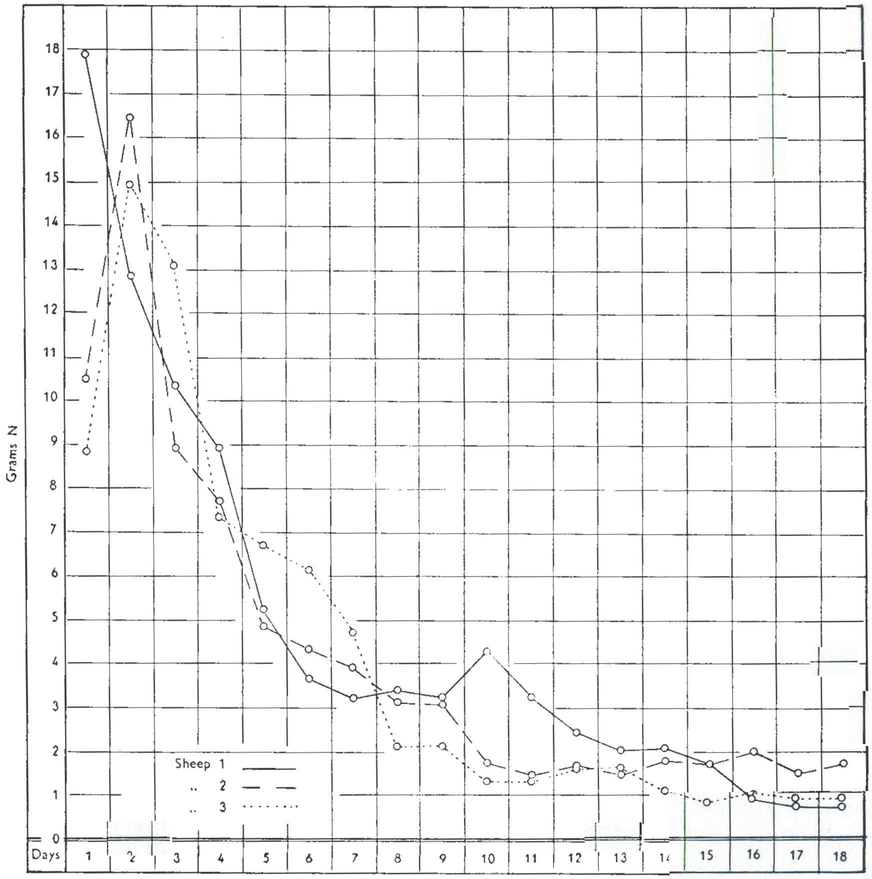
Dextrinized starch	73·0
Agar	20·0
Cod liver oil	2·0
Bone ash(¹)	3·0
Salt	2·0
TOTAL	100·0

(¹) Bone ash contains 42·5 per cent. Ca and 17·04 per cent. P.

Experimental Results.

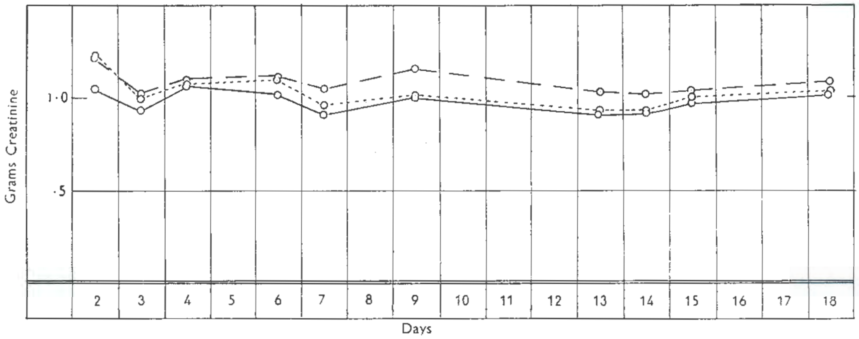
Three of the sheep which had been kept for a month on an exclusive lucerne ration of 14 per cent. protein, were put on the nitrogen-free ration and urine collections made daily. The curves representing the total daily nitrogen excretion are shown in graph 1. The low figures obtained with sheep 2 and 3 for the first day are undoubtedly due to the small quantities of urine voided, probably as a result of the change in quarters. It is apparent from the graphs that there is a sharp decline in total nitrogen excretion from the second day to approximately the eighth day. From then onwards the decline in the curve is considerably reduced. From these results it appears as if sheep 3 has attained its endogenous level round about the fourteenth day and sheep 2 at the eleventh day, while sheep 1 only reached a steady output of nitrogen from the sixteenth to the seventeenth day. Sheep 1 was the best eater of the three, which fact may lead one to suspect that the nutritional condition may be a factor in determining the length of the Nitrogen low period before the endogenous level is actually attained.

Graph 1.

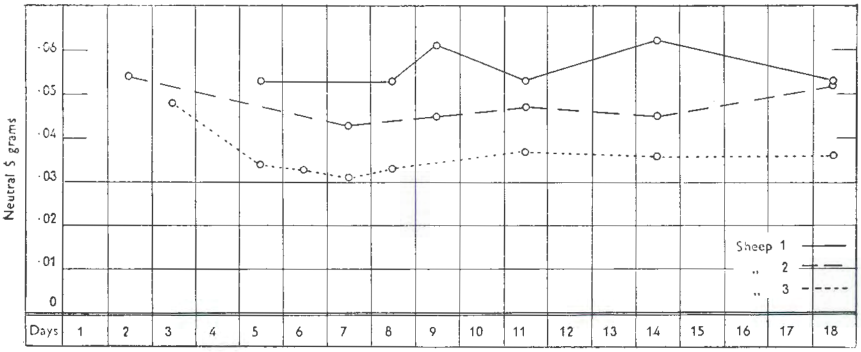


In graph 2 the daily excretion of creatinine for each sheep is given. It is evident that the excretion of this nitrogenous end-product is not influenced by the distinct decrease in total nitrogen during the first eight days. There is a tendency with all three sheep to show a slightly elevated excretion of creatinine during the first two days, probably as a result of preformed creatine in the previous ration. From the third day, however, the excretion is very uniform for all three sheep and almost assumes a straight line. The neutral sulphur is very constant for sheep 2 and 3 but varies somewhat in sheep 1. The elevated points on the curve of sheep 1 at the ninth and the fourteenth day are unexpected, since the other four determinations are absolutely of the same magnitude.

GRAPH 2.

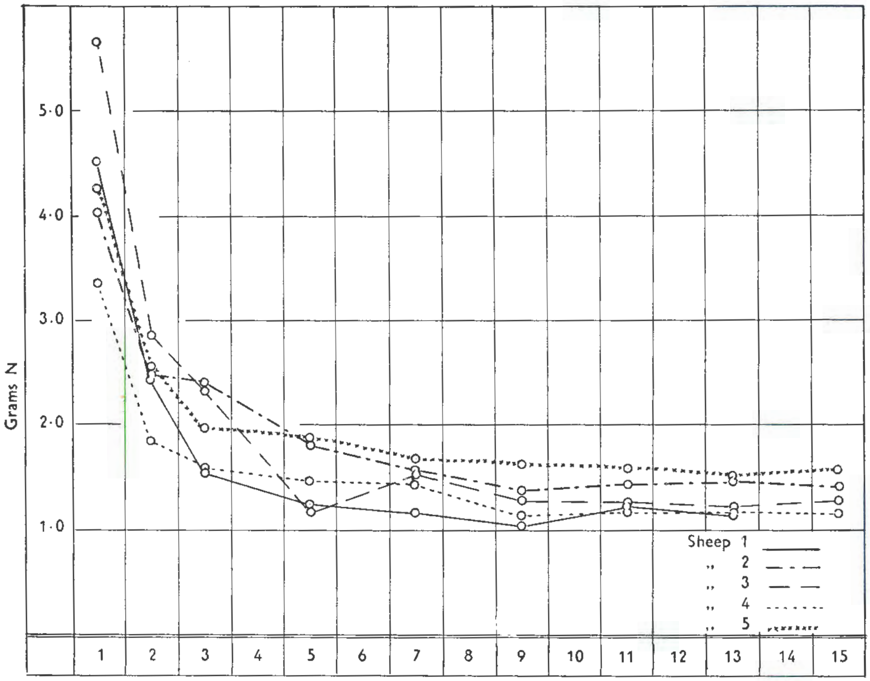


GRAPH 3.



In graph 4 are reproduced the results of four sheep which were kept for three months on an 8 per cent. lucerne protein ration supplemented by starch. The initial sharp fall in the daily nitrogen excretion occurred in this case during the first two days, while in the case of the 14 per cent. protein, it stretched over the first eight days. The endogenous level on the average for the four sheep seemed to be obtained after the sixth day. These results therefore seem to indicate that the deposit protein stores were decreased during the period of lower level protein feeding. At the 8 per cent. level of protein feeding, the period to reduce these sheep to their endogenous level was almost half that necessary to attain a constant excretion of nitrogen on a 14 per cent. protein level. This point strongly emphasizes the necessity of employing a standardized protein ration, before the endogenous nitrogen excretion is measured. It can readily be appreciated how a difference in level of protein may cause a difference in the ultimate endogenous N figures, due to the fact that the actual endogenous level is not attained. It seems, that this point may not only influence the reproducibility of the biological values of feeds, but also account for the lower endogenous Nitrogen figures at the end of a series of experiments, as suggested by Ashworth (1935). However, Ashworth's explanation that the lowered endogenous Nitrogen figures are caused by a reduced reserve protein due to continued Nitrogen-free feeding, is not supported by our findings. It is of course recognized, that just as the basal metabolism may be reduced through prolonged captivity so may the endogenous N be influenced. But under ordinary conditions of determining the biological value of feeds, the period of confinement does not seem to be long enough to effect such a reduction.

GRAPH 4.



Four sheep were taken off a lucerne ration and transferred to a Nitrogen-free ration, on which they were kept for 20 days. They were then put on an 8 per cent. lucerne protein ration for 20 days, on a similar ration supplemented by cystine for the same period and ultimately for 25 days on a nitrogen-free ration. The endogenous nitrogen per Kg. weight for the four sheep was as follows: ·036, ·048, ·035 and ·033 grm. respectively at the beginning of the experiment and ·033, ·040, ·033 and ·033 grm. at the termination of the experiment. These results certainly support the constancy of the endogenous nitrogen metabolism. The lowered endogenous nitrogen noted by Ashworth (1935) is therefore in all probability due to the fact that the endogenous level was in reality not attained at the start of the experiment on account of too short a preliminary N free period, the length of which, as has been shown, being in a great measure determined by the level of protein intake.

Our average figure of ·035 grm. endogenous N per Kg. weight agrees very well with that reported by Satola but is much lower than the value of ·063 reported by Hutchinson and Morris (1936).

In Table 2 is given the distribution of the nitrogenous and sulphur end-products in the urine of sheep kept on a nitrogen free ration. The creatinine nitrogen constitutes a larger portion of the total nitrogen as the sheep reached the endogenous level. During the first day or two after the sheep have been taken off the protein ration, the creatinine only makes up 2 per cent. of the total nitrogen excretion. When these animals have attained their endogenous level the creatinine as shown in Table 2 makes up approximately 37 per cent. of the total nitrogen excretion. The diminished nitrogen excretion is therefore in greater part due to the cessation of exogenous catabolism of protein, and consequently to a decreased excretion of urea which is the chief end-product of exogenous protein catabolism. Smuts (1935) has shown that as the size of the animal increases, the creatinine assumes a larger proportion of the endogenous nitrogen. In mice it is approximately 5 per cent., in guinea pigs 7 per cent., in rabbits 14·3 per cent. and in pigs 14·3 per cent.

TABLE II.

Distribution of Urinary end-products of Sheep on N-free Ration.

Animal No.	Weight Kgrms.	Endog. N Grm.	Creatinine N Grm.	Creatinine coefficient.	Creatinine N Per cent. of End N.	Total S. Grm.	Neutral S. Grm.	Ratio N/S.
1	33	1·10	0·41	12	44	0·129	0·055	8·5
2	35	1·40	0·41	12	29	0·203	0·056	6·9
3	36	1·29	0·48	13	37	0·216	0·069	6·0
5	42	1·19	0·44	11	37	0·145	0·039	8·2
Average	—	—	—	12	37	—	—	7·4

The creatinine coefficient expressed in terms of Schaefer's definition, namely, milligrams creatinine nitrogen per Kg. weight, is recognized by physiologists as an index of muscular mass and development. For this reason it is not a very uncommon phenomenon to find a variation in this index, depending on the fitness of an animal. The creatinine coefficient, obtained for sheep in this study, namely 12, falls in line with the figures reported for man and pig. For smaller animals like the mouse, rabbit and guinea pig the creatinine coefficient is larger. For guinea pigs this figure as reported by Smuts (1935) is approximately 14, for mice 30 and for rabbits 17.

The ratio of sulphur to total nitrogen excretion is often taken as an index of the type of catabolism which is proceeding in the body. Under fasting conditions where tissue catabolism takes place this ratio is approximately 16. In as much as our figure of 7·4 is much lower, it affords additional evidence that our animals were strictly on a non-tissue catabolizing level.

The maintenance requirement of an animal for protein seems to be determined by the inevitable and constant loss of essential nitrogenous constituents of the tissues as a result of the endogenous catabolism. Consequently there is a certain amount of food protein or other available sources of nitrogen needed to replenish this loss of nitrogen in order to maintain the normal functioning of tissues and the life of the animal. This constant basal need of protein is comparable to the basal needs of energy, and forms the foundation on which are superimposed the protein requirements for growth, lactation and other functions.

From the above reasoning it is evident that the maintenance requirement of nitrogen of an animal may, therefore, be measured by the total nitrogen excretion in the urine of an animal kept for a long enough period on a nitrogen free ration to establish its endogenous catabolism. For the four sheep under investigation an average figure of ·035 grms. nitrogen per Kg. weight was obtained. However, in later determinations on 10 sheep, it was found that values for the endogenous nitrogen vary from ·035 grms. to ·048 grms. per Kg. weight, with an average value of ·041. Taking the latter figure as more representative, it would mean that the protein required for maintenance per Kg. weight is equivalent to ·256 grms. This value naturally represents protein actually utilized by the body. Therefore to convert it to digestible crude protein, the biological value should be taken into account. If, as in the case of lucerne, the biological value for sheep is 60, then the digestible protein requirements become $\cdot 256 \times \frac{100}{60} = \cdot 43$ grms., which is equivalent to 19 grms. digestible protein per 100 lb. weight. The corresponding amount of dietary protein would be larger than this, and would be greater the greater its wastage in metabolism and hence the smaller its biological value. Consequently, when a protein feed with a lower biological value than that of lucerne is utilized, a proportionately larger amount of digestible protein is necessary. However, if we allow a fair margin of safety and assume an average biological value of 50 for maintenance, then the maintenance requirement in terms of digestible protein for a 100 lb. sheep becomes 23 gms. This figure is somewhat lower than 27 grms. digestible protein, proposed by Armsby (1917) and almost three times lower than that proposed by Henry and Morrison (1923).

Smuts (1935) adapted the Brody equation $Q=70{\cdot}4m^{\cdot 734}$ for the prediction of the basal metabolism of mature mammals to a determination of the protein requirements by the use of his ratio 12·5 mgm. protein per calorie of basal heat.

The equation then became $P={\cdot}88\ M^{\cdot 734}$, where P is the day's requirement of protein expressed in grams. The protein requirements obtained in this way do not refer to dietary protein or digestible protein but to protein actually utilized. Assuming therefore a biological value of 50, the digestible protein requirements per 100 lbs. live weight for sheep become 29 grams. This predicted figure compares favourably with the determined value of 23 grams digestible protein. It appears, therefore, that this equation can be applied successfully to the determination of the maintenance protein requirement of sheep. On the other hand, by reversing the calculation, the basal energy needs of sheep may be predicted with equal ease. Hence 29 grms. digestible protein having a biological value of 50, is equivalent to 14·5 grms. utilizable protein. By applying the Smuts equation for the relationship between endogenous nitrogen and basal metabolism, namely 12·5 mgm. P=1 Calorie basal heat, a figure of 1160 Calorie basal heat per 100 lb. sheep is obtained. This figure agrees well with the reported data on the basal metabolism of sheep. Ritzman and Benedict (1930) report a value of 1260 Calories per 100 lb. sheep. This figure was obtained by deducting 17 per cent. from their standard metabolism, and may therefore be slightly high. Rubner's Standard of approximately 1040 Calorie is slightly below our predicted value, while Krog's standard of 1248 Calories per 100 lbs. is slightly higher. However, the differences are so small that the relationship may be successfully utilized in predicting the basal energy needs of sheep.

Summary and Conclusions.

By means of a nitrogen free diet the time period for the attainment of the endogenous nitrogen level has been established in sheep. It appears that the preliminary protein period is a determining factor in the length of the nitrogen free period. Sheep on a 14 per cent. protein were reduced to endogenous level in 14 to 15 days, while sheep kept on an 8 per cent. protein attain the endogenous level from the sixth to the eighth day of nitrogen-free feeding.

The partition of the urinary end products on a nitrogen free ration is discussed and special consideration is given to the maintenance requirement of sheep. It has been shown in this study, that the maintenance requirement of protein of a 100 lb. sheep is less than advocated in the usual standards.

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