CCXXXVIII. THE DIGESTIBILITY OF DIETARY PROTEIN IN THE RUMINANT.

I. ENDOGENOUS NITROGEN EXCRETION ON A LOW NITROGEN DIET AND IN STARVATION.

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IN studying the digestibility of food protein the normal procedure is to analyse the food for its content of N and to feed a constant amount to experimental animals. After a preliminary period of such length that the faeces can be presumed to represent the experimental ration, the faeces are collected and analysed for a period of 7–10 days. In certain experiments markers have been used to distinguish the faeces of this period from that of the preceding and following periods. The percentage of the ingested nitrogen *not* appearing in the faeces is called the “coefficient of digestibility of protein”.

This definition assumes that all the faecal N is derived from the ingested N. Previous work [Schneider, 1934] has shown, however, that the faecal N can be divided into three fractions, (a) a constant fraction found when the animal is fasting, (b) a fraction dependent on the amount of both total dry matter and of indigestible dry matter in the diet and (c) a fraction dependent on the nitrogen content of the diet.

METABOLIC FAECAL NITROGEN.

Considerable work has been carried out on the metabolic faecal N of the one-stomached animal and its relation to the food intake. Mitchell [1924] concluded that the metabolic faecal N of young growing rats was directly proportional to the food intake. Whilst he recognized the presence of a constant amount of endogenous N he considered it quantitatively of no importance and recommended therefore that the metabolic faecal N on a normal diet should be calculated as faecal N/100 g. food intake.

Boas-Fixsen and Jackson [1932], working with adult male rats and consequently with a much lower food intake, found that the endogenous faecal N was roughly constant for any particular rat and was by no means directly proportional to the food intake. They considered that the food intake should not be taken into account in estimating the metabolic faecal N, in the calculation of the biological value of proteins for maintenance.

Schneider [1934] made a statistical analysis of the results obtained in Mitchell's laboratory over a number of years. He confirmed Boas-Fixsen and Jackson's finding that, in an animal eating its maintenance requirements or less, the constant fraction of the metabolic faecal N represented a comparatively large fraction of the total faecal N. Calculation of the metabolic faecal N on the assumption that it is directly proportional to the food intake would thus involve an error as great as if the food intake were not taken into account at all. Where, however, the food intake is above the maintenance requirements of the animal

the metabolic faecal N may be taken as directly proportional to the food intake.

Voit [1932], working with adult dogs, found that the constant fraction was by no means negligible. He concluded from his experiment that fat exercised less effect on the metabolic faecal N than carbohydrate and protein. Mitchell [1934], however, could not confirm Voit's results, but found that, irrespective of the type of food ingested, the metabolic faecal N bore a constant relationship to the food intake.

With ruminants, various investigators have attempted to obtain a figure for the metabolic N. Morgen *et al.* [1914] fed to sheep a ration consisting of extracted straw, starch, sugar and oil. In most of the experiments blood albumin was added to increase the palatability of the ration. It was thought that, since the albumin would be completely digested, it would have no effect on the faecal N excretion. This fact had previously been noted with one-stomached animals by Mendel & Fine [1912] and Underhill & Goldschmidt [1913] and was later confirmed by Mitchell [1924]. Morgen *et al.* observed 0.51 g. of faecal N excreted per 100 g. of dry matter intake. Later, Titus [1927] investigated the endogenous N excretion of steers, the rations consisting of varying amounts of alfalfa and cello-mass (dry paper pulp). He noted that the faecal N varied markedly with the moisture content of the faeces. By calculating all his results on an assumed 80% moisture content, he found, by extrapolation, that the metabolic faecal N was 0.34 g. per 100 g. dry matter intake.

In the present series of experiments an attempt has been made to obtain a value for the metabolic faecal N of goats and sheep by feeding N-free diets and rations of low N content.

I. *The effect of a nitrogen-free diet on the faecal nitrogen excretion.*

Four goats and four sheep were used, the experiments being divided into three periods, (1) starvation, (2) low food intake, (3) high food intake. The energy was provided as starch and olive oil. The olive oil comprised 2% of the high food intake and 2-9% of the low food intake diet. To provide roughage, 12-40% sawdust or paper was incorporated in the diet in the experiments with low food intake. In the experiments with high food intake the diet contained 20% of sawdust. 2-5% of a salt mixture was given in all diets and 30 drops of adexolin daily to provide vitamins A and D. In the high food intake experiments the diet was calculated to give the maintenance energy requirements from Benedict & Ritzman's [1926] figures for sheep. The N content of the diets was 0.02% except in three experiments with high food intake, in which there was 0.48% caseinogen and 0.32% urea N. These diets contained rather less indigestible carbohydrate than the average normal diet, since it was not possible to give any more cellulose. The total food intake on the high food intake diet was therefore less than normal.

It was necessary to feed the diets by a bottle and stomach tube, since the animals would not consume a N-free diet of their own accord. About half the paper of the low food intake diet was, however, voluntarily consumed by the goats. It was not possible to perform satisfactory experiments on the sheep with the high food intake diet, since the animals became ill and eventually died. Apparently the fibre accumulated in the rumen instead of being passed on to the true stomach. The experiments lasted 6-14 days. All the starvation experiments lasted 7 days, during which water was given *ad lib.* as in the other experiments. The urine and faeces were collected daily. A preliminary period was given, usually 3 or 4 days, till the animals reached a constant N excretion.

There was some doubt as to the validity of results obtained by such a drastic method of feeding. They might be too low, if the fibre were to accumulate in the rumen instead of being regurgitated and then passed into the true stomach. This occurred when the sheep were given the high food intake, so that the faecal N was actually lower than with the low food intake, while the weight of faeces was also smaller. Nevertheless, it was possible to keep the goats up to 14 days on the high food intake and 21 days on the low food intake without any ill effects.

However, in order to obtain more definite evidence on this point, an attempt was made to get some of the animals to consume a N-free diet voluntarily. After considerable difficulty it proved possible to feed to three goats a diet composed of starch, sugar, sawdust and a small quantity of straw. The amount of straw was too small to have affected the results even if it contained indigestible N. The details of these diets are shown in Table I. 10 g. of salt and 5 ml. of cod-liver oil were given to each animal daily.

Table I. *Daily rations.*

Animal	Body weight kg.	Starch g.	Sawdust g.	Oat straw g.	Sugar g.
1	18.5	114	10	101	10
2	14.2	111	17	87	10
3	24.0	154	14	95	10

The results of the two series of experiments are shown in Fig. 1, where $\frac{\text{Faecal N}}{(\text{Body weight})^{\frac{2}{3}}}$ is plotted against $\frac{\text{Food intake}}{(\text{Body weight})^{\frac{2}{3}}}$ in order to compensate for the difference in size between the different animals. The general form of the graph is in agreement with that obtained with the one-stomached animal. The

Fig. 1. Faecal N excretion of goats and sheep.

constant fraction is a fairly large proportion of the total metabolic faecal N. The points corresponding to the animals which ate voluntarily (denoted by crosses) lie in the centre of the distribution of points. It appears therefore that forcible feeding did not affect the general trend of faecal N excretion.

The line of the graph corresponds to a metabolic faecal N of 0.50 g. per 100 g. dry matter food intake at the high food intake level. As explained above, this

point on the graph corresponds to a food intake which is below the normal. At the normal food intake the value would be 0.45, which is lower than Morgen's figure of 0.51 for the sheep. The metabolic faecal N of the goat and sheep appears then to be relatively high, being in fact about double the endogenous urinary excretion.

II. *The effect of fibre on the metabolic nitrogen excretion.*

The effect of indigestible non-nitrogenous substances on endogenous faecal N excretion has been studied with rats [Mendel & Fine, 1912; Mitchell, 1924]. It was found that there was an increase in faecal N which depended on the amount of roughage ingested. With the ruminant, fibre is a very essential part of the ration. It was important, therefore, to discover whether the effect of fibre on the metabolic faecal N excretion of the ruminant was similar to that found with rats.

Two series of experiments were carried out on goats. Three animals which had been forcibly fed on the low food intake diet in the first series of experiments were given a further experimental period in which paper was substituted for half the starch. The total food intakes were slightly different in both experiments, but this was insufficient materially to affect the results. The experimental periods were of 6-8 days' duration.

It will be seen that the fibre invariably increased the metabolic faecal N (Table II).

Table II. *The effect of fibre on the faecal nitrogen excretion.*

	Body weight kg.	Diet without fibre		Diet with fibre		Faecal N with- out fibre (g.)/ 100 g. dry food intake	Faecal N with fibre (g.)/ 100 g. dry food intake
		Starch etc. (dry) g.	Paper (dry) g.	Starch etc. (dry) g.	Paper (dry) g.		
Goat							
4	63	366	0	323	63	0.505	0.585
5	23	147	0	135	23	0.490	0.550
6	39	262	0	226	72	0.410	0.450

A more accurate series of experiments was attempted on three further goats. The periods of collection in these experiments were 10 days, exclusive of the preliminary period. Paper and cellophane were used as the source of fibre and it was found possible to make a greater change in the fibre content of the two diets.

It will be seen (Table III) that animal 6 had a completely N-free diet while the small quantity of maize and oats in the diets of the other two animals was not sufficient to affect the results even if the digestibility had fallen slightly in the high-fibre period.

Table III.

	Body weight kg.	Low-fibre diet			High-fibre diet		
		Oats and flaked maize g.	Paper g.	Starch etc. g.	Oats and maize g.	Paper and cellophane g.	Starch etc. g.
Goat							
5	23	30	10	173	30	101	82
3	24	30	10	114	30	82	48
6	38	0	23	236	0	126	144

As in the first series of experiments, the additional fibre increased the metabolic faecal N of each animal (Table IV). It is possible that the large increase noted would have been reduced on a higher food intake. This, however,

Table IV.

Goat	Faecal N on low-fibre diet g.	Faecal N on high-fibre diet g.	Difference in fibre content of 2 diets g.	Increase in faecal N/100 g. fibre g.
5	1.10	1.84	75	1.77
3	0.87	1.09	67	0.59
6	1.24	1.78	100	0.97
			Mean	1.11

proved impossible to investigate, since the animals did not maintain their appetites when more food was given.

Since the effect of fibre on the metabolic faecal N was so great it seemed possible that the true digestibility of straw, which contains 35% of fibre, might be higher than has been generally supposed. If this were so, straw might be used as roughage in determining the metabolic faecal N of the cow, which will not consume cellophane or paper. Two of the goats which ate voluntarily, in a previous experiment (p. 1684), the diet containing starch, sugar, sawdust and a little straw, were therefore given a diet composed only of straw with the same quantity of salts and cod-liver oil.

It appears from the results (Table V) that the true digestibility of straw, using the goat as experimental animal, is 100%. It must, however, be remem-

Table V. *The digestibility of straw.*

Goat		Daily intake g.	N ingested g.	Faecal N g.	Difference in straw intake g.	Difference in fibre intake g.	Difference in faecal N g.	Difference in met. faecal N (due to difference in fibre intake) g.
1	Low-straw	114 starch + sugar 10 sawdust 101 straw	0.31	1.16	—	—	—	—
1	High-straw	213 straw	0.65	1.55	112	34	0.39	0.38
2	Low-straw	111 starch + sugar 17 sawdust 87 straw	0.25	1.18	—	—	—	—
2	High-straw	248 straw	0.70	1.64	161	47	0.46	0.52

True digestibility of straw 100% with both animals.

bered that the experimental error involved in the measurement of small differences in faecal N is large. Also the number of experiments is small. Nevertheless it can be concluded with safety that the true digestibility of straw is high. This would explain the remarkably close agreement between the results of the present series of experiments and those of Morgen, who fed extracted straw in the diet.

III. *The metabolic faecal nitrogen excretion of the cow.*

Since, in order to determine the biological value of food proteins in the ruminant for maintenance or production, the cow or steer is the animal most generally used, it seemed advisable to obtain values for excretion of endogenous origin for the cow instead of assuming that the result for one species, the goat, would hold for other ruminants. For this purpose, two cows A and B were used, the low-N ration fed consisting of straw, maize and starch (Table VI).

Table VI.

	Starch equivalent lb.	N content g.
1 lb. flaked maize	0.84	7.25
12 lb. straw	2.52	17.40
2.25 lb. starch	2.25	1.02
200 ml. linseed oil	1.00	0.00
	6.61	25.67

Straw was used instead of paper or cellophane, since neither of these materials was eaten in sufficient amount to satisfy the needs of the animals for bulk in the ration. Since, however, straw had shown a remarkably high digestibility with goats, and since a further experiment with the two cows showed that the faecal excretion after ingestion of only 16 lb. straw did not differ from the excretion on the above diet, it seems safe to assume that the faecal N excreted has been very close to the actual metabolic faecal N. It can also be concluded that the maize had a digestibility very similar to that of straw. Further confirmation appears from the fact that the apparent digestibility of maize is over 90 %, which will give a true digestibility of approximately 100 %.

Preliminary periods of about 6 days were given with both animals before collection and analysis, while the actual experimental periods were 6 days for cow A and 9 days for cow B. A third experiment was performed on an animal (cow C) which had a rumen fistula. The straw was eaten voluntarily, while starch which formed the concentrate part of the ration was put into the rumen by way of the fistula. The cow was fed 2 lb. starch and 14 lb. straw. The results of all three experiments are shown below (Table VII). The food in all experiments contained 10 % moisture.

Table VII.

Cow	Daily food intake lb.	Daily faecal N g.	Faecal N/100 g. dry food intake
A	15.5	29.9	0.48
B	15.5	26.0	0.42
C	14.0	25.0	0.44
		Mean	0.45

It appears that the faecal N of the cow like that of the goat and sheep is very high, the amount per 100 g. food intake being about double that of the one-stomached animal. The large quantity of indigestible carbohydrate in the diet of the ruminant may be the cause of this.

In order to find out the size of the constant fraction of the metabolic faecal N of the cow, cows A and B were starved for 7 and 9 days respectively (Table VIII). The faecal N of cow A became steady after 3 days and averaged 5.9 g. daily. The faecal N of cow B became steady after 2 days and averaged 10.1 g. daily. Two further cows C and D were starved for 9 and 12 days, while collections were made on the last 6 and 9 days respectively. The faecal N of cow C was 3.9 and of cow D 8.1 g. daily. If it had been possible to continue the starvation longer it is probable that the faecal N would have fallen still further, as in Carpenter's [1927] experiment on steers. During the period under observation, however, it remained fairly steady, and, since this period is one with a negligible food intake (the food which remained in the rumen when the fast commenced was slowly passed through the alimentary canal) it probably gives a fairly accurate figure for the constant fraction of the metabolic faecal N. It appears that the constant

Table VIII. *Daily faecal nitrogen excretion during starvation.*

Day of fast	Faecal N (g.)			
	Cow A	Cow B	Cow C	Cow D
1	25.91	26.67	32.00	20.48
2	17.51	15.50	22.84	9.56
3	14.64	15.14	6.68	22.36
4	6.39	10.50	3.98	5.93
5	5.12	11.80	4.25	9.13
6	4.93	5.16	5.51	9.78
7	6.53	10.61	2.07	8.03
8	—	8.86	2.50	7.50
9	—	12.95	4.84	8.96
10	—	6.46	—	8.72
11	—	—	—	3.65
12	—	—	—	11.59

fraction is specific for the different animals. The mean faecal N for all four cows during starvation was 7 g. daily, about one-quarter of the metabolic faecal N on a normal ration.

THE ENDOGENOUS URINARY NITROGEN.

During the above experiments a detailed study of the endogenous urinary excretion was also undertaken, together with a study of the fasting catabolism.

I. *The fasting catabolism of ruminants.*

Much work on fasting protein catabolism has been done in the past using one-stomached animals, experiments which, up to 1920, have been admirably reviewed by Cathcart [1920]. After that date little of note was produced until the publication of Boy [1934]. In a series of experiments, using rabbits, pigs and dogs as experimental animals, Boy claimed that the increase in N excretion during a fast following a N-free diet was composed in the main of urea, ammonia and creatine. The results, however, do not warrant the conclusions drawn, since whereas in some cases an increase in ammonia excretion was definite, in others no increase or even a decrease was found. Boy concluded that N metabolism in starvation is merely enhanced protein catabolism, similar to that found after ingestion of protein. It will be shown that the metabolism during a fast is very similar with all species of animals, both ruminant and one-stomached, and the conclusion that this can be reproduced by the feeding of protein appears to be correct.

In the past little attention has been paid to the N metabolism of the fasting ruminant. Grouven [1864] noted a daily loss of 50–60 g. urinary N in the cow. Various workers have determined the amounts of one or two constituents in the urine of fasting ruminants [Prayon, 1910; Palladin, 1924; Sjollem & van der Zande, 1923], but little of any importance was published until the detailed study with steers by Benedict & Ritzman [1927]. The full results of this investigation published by Carpenter [1927] showed that the N excretion per kg. body weight in 24 hours was 0.064–0.075 g. at a maximum. Expressed as percentages of the total N the urea N rose markedly whilst the amino-acid and hippuric acid N fell, the ammonia N remaining more or less unchanged. No ketonuria was noted, a fact which agreed with the findings of Sjollem & van der Zande [1923]. From the results Carpenter concludes that the effect of the previous diet had not disappeared till the 4th to the 6th day of the fast, from which day the true fasting base line can be taken.

Table IX. *Fasting catabolism of ruminants.*

Day of fast	Total N g.	Urea N g.	Ammonia N g.	Amino-acid N mg.	Preformed creatinine g.	Creatine g.	Sulphur g.	S : N
Cow A. 417 kg. Low-N diet.								
1	14.95	4.31	5.90	0	3.97	0.74	2.31	1 : 6.5
2	19.94	7.21	4.94	870	5.40	1.80	2.01	1 : 10.0
3	19.16	8.61	2.47	110	3.16	3.44	1.08	1 : 17.7
4	28.13	16.75	2.15	140	5.06	1.59	1.20	1 : 23.4
5	27.32	17.77	1.83	40	3.68	1.92	1.15	1 : 23.7
6	49.45	29.24	2.14	270	5.52	3.18	2.16	1 : 22.9
7	54.12	32.36	2.69	370	5.65	3.09	2.24	1 : 24.1
Cow B. 453 kg. Low-N diet.								
1	19.71	4.19	5.17	320	4.73	0.25	1.26	1 : 15.7
2	34.06	20.68	2.12	250	6.39	0.90	2.52	1 : 13.5
3	42.67	28.50	2.10	260	5.95	1.78	3.06	1 : 13.8
4	41.88	28.70	1.40	240	5.45	1.92	2.88	1 : 14.6
5	34.98	21.94	2.42	150	5.99	1.60	1.13	1 : 30.9
6	32.21	19.13	2.47	340	6.12	1.90	5.04	1 : 6.4
7	40.76	24.58	3.95	300	6.52	2.03	2.47	1 : 16.5
8	46.71	26.07	7.48	340	6.23	2.40	3.42	1 : 13.7
9	43.96	27.43	4.57	150	5.69	1.72	2.59	1 : 17.0
10	42.78	23.05	5.55	440	5.31	1.24	2.58	1 : 16.6
Sheep A. 37 kg. pasture.								
			mg.		mg.	mg.		
1	7.50	—	—	—	—	—	—	—
2	5.36	4.49	130	20	330	150	—	—
3	7.35	5.88	90	30	540	250	—	—
4	10.85	8.46	120	20	750	130	—	—
5	6.28	5.11	120	10	—	—	—	—
6	5.15	—	—	—	—	—	—	—
Sheep B. 36.5 kg. pasture.								
1	—*	—	—	—	—	—	—	—
2	—†	—	—	—	—	—	—	—
3	15.22	12.20	300	140	1190	750	—	—
4	8.94	7.51	310	20	650	760	—	—
5	4.06	3.15	150	0	—	—	—	—
6	5.63	—	—	—	—	—	—	—
7	7.16	6.28	480	30	—	—	—	—
Goat 1. 62 kg. Low-N diet.								
1	5.07	2.72	1000	30	740	140	—	—
2	7.31	5.47	410	40	760	490	—	—
3	7.30	5.31	120	50	710	200	—	—
4	7.35	6.31	200	20	710	630	—	—
5	7.02	5.55	330	—	—	—	—	—
6	7.20	—	—	—	—	—	—	—
7	5.38	3.33	1040	40	—	—	—	—
Goat 2. 24 kg. Low-N diet.								
1	2.05	0.95	40	10	390	0	—	—
2	3.76	2.69	90	30	520	60	—	—
3	3.67	2.84	120	40	390	70	—	—
4	1.73	1.29	80	20	220	150	—	—
5	1.15	—	150	—	—	—	—	—
6	4.58	—	—	—	—	—	—	—
Goat 3. 40 kg. Low-N diet.								
1	2.83	2.08	190	20	—	—	—	—
2	5.56	2.88	160	40	1060	170	—	—
3	6.25	4.33	270	60	510	90	—	—
4	4.47	3.26	220	30	670	360	—	—
5	7.62	5.19	340	—	—	—	—	—
6	5.05	—	—	—	—	—	—	—
7	4.36	2.18	210	40	—	—	—	—

* First day of fast.

† First day of fast. No urination on first two days of fast.

In the present investigation the results are shown of the fasting catabolism of goats, sheep and cows following a low-N diet. It will be seen (Table IX) that starvation following a low-N diet causes an increase in urinary N excretion. With cow A the base line has been reached on the 6th day of the fast, whereas with cow B the level was reached on the 3rd day, although the previous diet was the same. With the goats and sheep the same marked rise in the output was noted. The daily N excretion per kg. body weight was 0.088–0.13 for the cows, 0.13 for the goats and 0.16 for the sheep.

Regarding the urinary partition, the most marked effects are to be seen in the increases in urea and creatine excretion. If, as Boy states, the fasting catabolism is merely an accelerated protein catabolism, the difference between the true endogenous level and the fasting level should be typical of protein metabolism. The sole difference between the two types of catabolism, endogenous and fasting, lies in the urea and creatine increases (Table X). A comparison of the differences between low and high protein diets shows that there is perfect agreement between the two types of metabolism, that of tissue protein and that of ingested protein.

Table X.

Period	Total N g.	Urea N g.	Ammonia N g.	Amino- acid N g.	Preformed creatinine g.	Creatine g.
Goat 1.						
Low-N	2.52	1.19	0.47	0.059	0.28	0.01
Starvation	6.93	5.24	0.42	0.035	0.26	0.16
Difference	4.41	4.05	-0.05	-0.024	-0.02	0.15
Goat 2.						
Low-N	1.40	0.56	0.22	0.038	0.18	0.02
Starvation	3.44	2.55	0.11	0.031	0.16	0.04
Difference	2.04	1.99	0.11	-0.007	-0.02	0.02
Goat 3.						
Low-N	1.66	0.70	0.12	0.045	0.33	0.02
Starvation	5.55	3.63	0.24	0.044	0.29	0.08
Difference	3.89	2.93	0.12	-0.001	-0.04	0.06
Cow A.						
Low-N	12.70	3.60	2.67	0.39	1.03	0.17
Starvation	39.70	24.00	2.20	0.20	1.33	0.65
Difference	27.00	20.40	-0.47	-0.19	0.30	0.48
Cow B.						
Low-N	20.90	6.80	4.12	0.63	1.92	0.33
Starvation	40.60	24.40	4.22	0.28	1.60	0.49
Difference	19.70	17.60	0.10	-0.35	-0.32	0.16

Much work has been done on the sulphur excretion of the one-stomached animal during starvation. This has been collected and reviewed by Kahn & Goodridge [1926]. Discussing the N : S ratio in the urine, the authors state the old view that it is the result of catabolism of muscular tissue. The results do not appear to justify this statement completely, since the N : S ratio of muscle is about 16, whilst the N : S ratio of the urine during starvation is about 14. Terroine & Razafimahery [1935] have recently investigated the subject of S catabolism and have divided total starvation, following protein starvation, into three periods: (a) the first, in which a very marked but transitory increase in the N : S ratio occurs followed by as marked a fall—this is stated to be the result of partial catabolism of tissue proteins; (b) the second period, one in which the N : S ratio slowly decreases; a period in which, according to Terroine, all the

tissues are being catabolized; (c) the third and premortal period, which shows marked increases in the output of urinary N and S, but a decrease in the N : S ratio of the urine.

With the ruminant the only work of note on S excretion during starvation is that of Carpenter [1927]. He found a constant increase in the N : S ratio as the fast progressed and in some cases obtained a figure of 60.

In the present experiment cow A showed a progressive increase in the N : S ratio, from 6.5 to 24.1, whereas cow B, except for days 5 and 6, showed a steady ratio throughout the experimental period. The significance of these results cannot be stated, but it is interesting to note that when the endogenous N and S outputs are subtracted from the starvation levels, the N : S ratio of the difference is very close to that of muscle tissue.

At present it is impossible to compare either these results or those obtained by Carpenter with Terroine's, since the period corresponding to Terroine's first period may be very much longer in the cow, owing to the large size of the animal. It seems essential that a fast of much longer duration be carried out with the cow to determine the exact significance of the S excretion.

It is worthy of note that only with two of the goats was any ketonuria noted, and this only intermittently and to a very small degree. This, as in the experiments reported by Carpenter and Sjollem, would presuppose complete metabolism of the body fat.

The undetermined N was high, as Carpenter also noted, but tended to increase in total amount as the fast progressed, although the percentage amount decreased markedly. It may be assumed with safety that this fraction consists of the purine bodies, and it appears that the ruminant tends to retain these compounds by minimizing cellular catabolism during a fast.

From a survey of all the results it seems possible to conclude that the N catabolism occurring during starvation is, for the first 7-10 days, the result of an enhanced protein breakdown.

II. *The nature of endogenous nitrogen metabolism.*

Much work has been done on the nature of the nitrogenous material catabolized during a N-free regime, since Folin [1905] postulated exogenous and endogenous forms of protein catabolism. Three theories regarding endogenous catabolism have been brought forward. Sherman [1920], confirming the earlier experiments of Osborne and Mendel [1914], assumed that the effect of ingested protein was to retard endogenous catabolism. When the ingested protein was completely efficient (i.e. as regards essential amino-acids) the endogenous N excretion would be minimum. This theory is completely opposed to the idea of a constant endogenous catabolism. Mitchell [1924] could not confirm the results of Sherman, finding, on the contrary, that all his evidence tended towards the theory of a constant endogenous catabolism. After reviewing all the work on the "minimum" endogenous catabolism Mitchell [1930] concluded that the precursors of the metabolites in all urine were the non-protein N compounds of the tissues, "each of which probably has some specific function to perform and is catabolized at varying rates in response to variations in the function it serves".

Terroine [1933] has reviewed the previous work and has concluded that the endogenous N metabolism arises from two sources. The urinary metabolites urea, ammonia and amino-acids, he states, arise from body protein catabolism, whilst the creatinine, creatine, allantoin and purine bodies arise from cellular catabolism.

Recently Roche [1934] analysed the tissues of rats dying from total starvation and from protein starvation and claimed that the water-soluble (extractable) N

was an intermediary stage in tissue anabolism or catabolism. From the decreases in lysine, tyrosine and tryptophan after protein starvation, Roche concluded that with a N-free diet there is a progressive partial breakdown of muscle protoplasm. In other words, the endogenous N is protein in origin, a theory closely allied to that of Terroine.

With ruminants various experiments have been carried out to determine the endogenous N excretion [Morgen *et al.*, 1911, 1914; Hart *et al.*, 1912; Steenbock *et al.*, 1915; Honcamp *et al.*, 1923; Scheunert *et al.*, 1922]. For the cow values ranging from 0.029 to 0.045 g. N per kg. body weight have been obtained whilst with sheep the values range from 0.024 to 0.072 g. N.

In the present experiments urine was collected daily from sheep and goats on the N-free diets and from cows on the low-N diet. In Fig. 2 the total urinary N

Fig. 2. Urinary N excretion of goats and sheep.

per kg. body weight is plotted against the food intake expressed as energy per kg. body weight. For the sheep the mean value per kg. body weight is 0.063 on the low food (energy) intake diet, for the goats 0.044 on the low and high food (energy) intake diets, and for the cows 0.039 on the low-N diet.

The urinary N partitions are shown in Tables X and XI. With all the animals as the food intake increases from the starvation level the percentages of urea and creatine decrease whereas the percentages of all the other constituents increase. From the results with the goats on the low and high energy intakes it appears that the true endogenous level of excretion has been attained on the low energy intake, except in the case of the creatine excretion, which is still slightly above the high energy level. With the cows the percentage urea excretion is slightly lower than with the goats and sheep, whilst the ammonia excretion is higher. Otherwise, with the three types of animals, the partition products expressed as a percentage of the total N output are very similar.

From a survey of the results it is impossible to state from what source the endogenous N has arisen, but experiments are in process to investigate this subject more fully.

SUMMARY.

1. Experiments were performed on goats, sheep and cows to obtain a figure for the endogenous excretion of N on a low-N diet.
2. The metabolic N excretion on a normal diet approximated 0.45 g. per 100 g. dry matter intake.

Table XI. *Partition products expressed as percentage of total N.*

	Urea N	Ammonia N	Amino-acid N	Preformed creatinine N	Creatine N
Starvation.					
Goat 1	75.6	6.1	0.5	3.7	2.3
2	74.2	3.2	0.9	4.6	1.2
3	65.5	4.4	0.8	5.2	1.4
				% creatine + creatinine N of total N	
Sheep 3	80.2	1.6	0.2		3.36
4	82.5	3.1	0.3		5.50
Cow A	60.3	5.6	0.5	4.7	2.3
B	60.0	10.4	0.7	5.5	1.7
Low food intake diet.					
Goat 1	47.0	18.7	2.34	11.3	0.5
2	40.2	15.8	2.70	12.5	1.5
3	42.4	7.1	2.70	19.6	1.2
Sheep 1	51.0	11.2	1.70	13.0	2.6
2	58.9	18.3	1.9	19.2	0.0
4	70.2	11.9	1.0	11.2	0.1
High food intake diet.					
Goat 1	47.1	11.0	1.7	11.1	0.4
2	29.1	11.4	3.8	20.9	0.0
3	39.2	16.8	1.5	20.5	0.0
Low-N diet.					
Cow A	26.9	19.9	2.9	11.4	1.8
B	32.1	19.4	3.0	11.3	0.6

3. The effect of fibre on the N excretion was studied with results closely approaching those with the one-stomached animal, the greater the amount of indigestible dry matter the larger the faecal N. When this factor is taken into account it has been shown that the N of straw, which has an apparent digestibility of 30 %, has a true digestibility of 100 %.

4. A study of the constant fraction, the N excretion during starvation, has been undertaken.

5. The urinary excretion of N during fasting and on a low-N diet has been examined. During the fast the N excretion per kg. body weight varied from 0.088 to 0.13 g. with the cows, and to 0.16 g. with sheep. Further evidence that the fasting catabolism is merely an accelerated protein catabolism has been obtained.

6. The endogenous N excretion per kg. body weight varied from 0.063 with sheep, 0.044 with goats, to 0.039 with cows.

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