Nitrogen balance studies with the milk-fed lamb

6*. Effect of starvation and realimentation

By D. M. WALKER

Department of Animal Husbandry, University of Sydney, Australia

1. Five male cross-bred lambs at the age of 3 weeks were starved for a period of 4 days. The utilization of nitrogen, energy and minerals was compared with the pre-starvation and post-starvation periods, when the lambs were given reconstituted dried whole cow's milk.

2. The mean loss in weight during starvation was 253 g/24 h. The gain in weight during realimentation was significantly greater than that in the pre-starvation period, when adjusted for differences in energy intake.

3. The urinary loss of N during starvation was 478 mg N/kg live weight 24 h. During realimentation the urinary N loss was greater than during the pre-starvation period. A higher urea excretion during realimentation was responsible for the increased urinary N.

4. Excretion of ammonia, creatine, amino acid and purine base N increased during starvation. Excretion of creatinine N remained constant.

5. The increase in total sulphur excretion during starvation was due mainly to an increase in the inorganic sulphate fraction. The ratio of N to S of 27:1 during starvation was much greater than would be expected from the katabolism of muscle tissue alone (ratio N to S = 16·2:1).

6. The titratable acidity and acetone excretion in the urine increased during starvation.

7. There was a marked reduction in the urinary excretion of chloride, potassium and sodium during starvation, an increase in the excretion of phosphorus, but little change in the excretion of calcium and magnesium. The loss of minerals was not closely related to the losses predicted from muscle tissue alone.

8. Pulse rate, respiratory rate, and rectal temperature all decreased during starvation. Heat production decreased after 1 day of starvation, but was relatively constant thereafter.

9. Blood urea concentrations did not change significantly during starvation.

10. The results for the lamb were in reasonable agreement with published results for the calf, except for the marked ketone acidosis and considerable increase in the excretion of purine N. The losses during starvation differed from those with the adult sheep in showing a relatively greater loss of body protein.

Alexander (1962) has demonstrated the fatal effect of a relatively short period of starvation on the newborn lamb, and has shown the importance of prenatal nutrition on the time of survival of the lamb under adverse environmental conditions. Starvation in the older lamb has not previously been investigated and is unlikely to occur under natural systems of rearing in which the lamb suckles the ewe. However, under a hand-rearing or early-weaning system of management, anorexia or diarrhoea may result in partial and occasionally total starvation. Although the biochemical and physiological consequences of starvation have been studied extensively in man and some other species, the experiments of Blaxter & Wood (1951) represent the only analysis of total starvation in the preruminant animal, not immediately postnatal.

The experiment described in this paper was concerned with the effect of a short period of total starvation and subsequent realimentation on the utilization of nitrogen by the milk-fed lamb. Measurements of other changes in metabolism were also made.

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Wherever possible the results were compared with those of Blaxter & Wood (1951) for the milk-fed calf, and with those of Morris & Ray (1939) for the adult sheep.

EXPERIMENTAL

Animals and their management

Five male cross-bred lambs ((Border Leicester ♂ × Merino ♀) × Dorset Horn ♂) were used. The lambs were born at pasture and at the commencement of the experiment, at about 7 days of age, their live weights ranged from 4·0 to 6·1 kg. Each lamb was housed separately in a metabolism cage and faeces and urine were collected as described previously (Walker & Faichney, 1964*a*).

The experimental period of 24 days was divided into a pre-starvation period of 14 days, a starvation period of 4 days and a post-starvation period of 6 days. Faeces and urine were collected during the last 6 days of the pre-starvation period and throughout the starvation and post-starvation periods. Faeces were bulked for analysis during each period. Urine was bulked at 2-day intervals in the pre-starvation period, but was subsequently collected daily for analysis.

The lambs were weighed daily 4 h after the morning feed. Pulse rate, respiratory rate and rectal temperature were measured daily at the time of weighing.

The closed-circuit respiration chamber of Alexander (1961) was used for the estimation of the heat production and respiratory quotient (RQ) of two lambs by indirect calorimetry during the starvation period. Measurements were made on the first lamb on each of the 4 days of the starvation period. Three estimations of 2·0, 1·5 and 1·0 h duration were made during the 1st day, with an interval of about 1·0 h between them. Two estimations, each of 2·0 h, were made on the 2nd day with an interval of 1·0 h between them, and one estimation of 2·0 h was made on the 3rd and 4th days. Measurements were made on the 2nd and 3rd days of starvation with the second lamb, each estimation being for 6·0 h.

The mean daily maximum and minimum temperatures in the animal house during the experimental period were 29° and 17° respectively.

Diets

The lambs were bottle-fed on reconstituted dried whole cow's milk containing 15 % total solids at an intake of about 130 kcal/kg live weight 24 h. Feed intake was increased after 7 days of the pre-starvation period to adjust for the increase in body-weight but was maintained constant thereafter. During starvation the volume of distilled water given was equivalent to that given as milk during the pre-starvation period. The lambs were fed twice daily at 07.00 h and 19.00 h, with milk or water previously warmed in a constant-temperature bath at 37°. Supplements of Aureomycin and a trace-element mixture were given daily as in a previous experiment (Walker & Cook, 1967). All lambs were dosed with 1 ml of a groundnut-oil solution of 100000 i.u. vitamin A acetate and 10000 i.u. ergocalciferol during the 1st week of the experiment.

Analytical methods

Diet and faeces. Total N was determined in the dried whole milk and in the moist faeces by the Kjeldahl method. Total sulphur in the dried milk and faeces was determined by ignition of the sample in a bomb calorimeter (Parr Instrument Company, 1960) followed by precipitation of the S as $BaSO_4$ according to the method of Benedict (1909). Faeces were dried to constant weight at 100°, and the dry-matter content of dried whole milk was determined by drying it to constant weight at 60° under reduced pressure. Ash and total fat in the dried milk and faeces were determined by the methods used in a previous experiment (Walker & Faichney, 1964a).

Urine. Urine was collected into a solution of 10 % (w/v) thymol in isopropanol (0·5 ml/100 ml urine). Samples (15 ml) of this urine were preserved in 2·5 % (v/v) HCl (1 ml) at −20° for the subsequent determination of calcium, magnesium and phosphorus. Total N, urea, ammonia, creatine, creatinine, uric acid, allantoin, purine bases, amino acids, total S, total sulphate, inorganic sulphate, ethereal sulphate and neutral S were determined by the methods used in a previous experiment (Walker & Faichney, 1964b). The following analytical methods were used for other constituents in the urine: Na and K (flame photometry); Ca and Mg (Carubelli, Smith & Hammersten, 1959); P and Cl (King & Wootton, 1959); titratable acidity (Hawk, Oser & Summerson, 1954); ketone bodies as acetone (Reid, 1960).

Muscle. Total N was determined on the wet muscle by the Kjeldahl method and dry-matter content by drying to constant weight at 100°. Total S was determined on the dry muscle by the method used for faeces. The muscle after drying was ashed in a muffle furnace at 450° and extracted with concentrated HCl as in the method of McCance, Widdowson & Shackleton (1938) for the determination of Na, K and P. Phosphates were removed on an ion-exchange column (Howard & Lawrie, 1956) before determination of Na and K by flame photometry. P was estimated by Allen's (1940) method after a sample of the acid extract of the ash had been heated with a few drops of concentrated H_2SO_4 to convert pyrophosphate into orthophosphate. Ca and Mg were determined on the acid extract of the ash by the method of Carubelli *et al.* (1959). Cl was determined by the method used by Dickerson & Widdowson (1960).

Muscle was prepared for the estimation of creatine by the method of the Association of Official Agricultural Chemists (1960) and creatine was determined by the method of Folin (1914). Protein N was determined by the Kjeldahl method after precipitation with trichloroacetic acid.

RESULTS

The lambs appeared normally active throughout the period of the experiment and drank both milk and water keenly at each feed. During the starvation period the lambs spent an increasing period each successive day lying in their metabolism cages, but did not develop a depraved appetite similar to that reported in starving calves by Blaxter & Wood (1951). The first three lambs were slowly reintroduced to food by giving one-quarter, one-half and three-quarters of their normal pre-starvation

allowance of milk at the first three feeds after starvation. No diarrhoea occurred. The last two lambs were given their full allowance of milk immediately after starvation, but there were neither digestive upsets nor diarrhoea.

Table 1. *Mean live weight, energy intake and live-weight gain or loss of the lambs in each period of the experiment*

	Pre-starvation			Starvation		Post-starvation		
Lamb no.	Live weight (kg)	Energy intake (kcal/ 24 h)	Live-weight gain (g/24 h)	Live weight (kg)	Live-weight loss (g/24 h)	Live weight (kg)	Energy intake (kcal/ 24 h)	Live-weight gain (g/24 h)
1	5·6	735	154	5·4	274	5·7	649	177
2	6·5	811	175	6·4	278	6·7	738	173
3	8·1	952	193	8·0	294	8·2	855	220
4	7·0	800	121	6·8	230	7·1	800	199
5	5·8	697	110	5·7	191	5·9	697	152
Mean	6·6	799	151	6·5	253	6·7	748	184

Table 2. *Mean values for lambs nos. 4 and 5 for the apparent digestibility coefficients of the diet and for the composition of the faecal dry matter*

	Pre-starvation	Starvation	Post-starvation
Apparent digestibility coefficient (%):			
Dry matter	98·1	—	98·0
Organic matter	98·2	—	98·4
Total N	97·1	—	97·1
Total fat	97·4	—	98·1
Fresh faeces excreted (g/24 h)	7·8	3·6	8·3
Faecal composition (%):			
Dry matter	33·6	33·7	37·2
Total N	7·2	7·8	6·3
Total fat	36·7	33·8	24·9
Neutral fat, free fatty acids	23·8	19·6	15·7
Soaps	12·9	14·2	9·2
Ash	9·5	11·2	25·1

Live-weight changes

Table 1 shows the mean live weights of the lambs, their intakes of energy and the daily live-weight changes in each period of the experiment. The gain in weight after starvation was greater, for four of the five lambs, than was observed in the pre-starvation period, even though the intake of energy was reduced for three lambs by giving a reduced volume of milk in the first three feeds after starvation to avoid the risk of diarrhoea or digestive upset. The loss in weight during starvation was not directly related to the live weight of the lambs and showed considerable variation between lambs. Nevertheless, the loss in weight during starvation was, for all lambs, greater than the gain in weight during the pre- and post-starvation periods, and was linear for the 4-day period. The total loss in weight was about 15 % of the pre-starvation live weight. Alexander (1962) showed that newborn lambs, born from ewes fed at a high plane of nutrition, and starved to death in still air at 23°, survived for 68 h and lost about 25 % of their body weight before death.

Digestibility of the diet and composition of the faeces

Table 2 gives mean values for the digestibility of the diet obtained with lambs nos. 4 and 5, which received the same intake of dry matter in the pre- and post-starvation periods. There were no significant changes in the apparent digestibility coefficients of any of the individual components between the pre- and post-starvation periods. The coefficients of digestibility were slightly higher than those obtained in an earlier experiment with this diet (Walker & Faichney, 1964c). Throughout the starvation period the lambs continued to produce faeces of similar composition and appearance to those produced in the feeding period. The mean values for the composition of the faecal dry matter during feeding and starvation are shown in Table 2.

Table 3. *Nitrogen balance of the lambs and the utilization of the apparently digested N*

Lamb no.	N intake (g/24 h)	Urinary N (g/24 h)	Faecal N (g/24 h)	N balance (g/24 h)	Apparently digested N retained (%)
		Pre-starvation (6 days)			
1	5·90	1·57	0·17	4·16	72·6
2	6·51	1·68	0·17	4·66	73·5
3	7·64	1·62	0·36	5·66	77·7
4	6·42	2·21	0·16	4·05	64·7
5	5·59	1·96	0·20	3·43	63·6
Mean	6·41	1·81	0·21	4·39	70·4
		Starvation (4 days)			
1	—	2·28	0·14	−2·42	—
2	—	2·76	0·08	−2·84	—
3	—	2·67	0·22	−2·89	—
4	—	3·35	0·09	−3·44	—
5	—	3·16	0·09	−3·25	—
Mean	—	2·84	0·12	−2·97	—
		Post-starvation (6 days)			
1	5·21	1·93	0·20	3·08	61·5
2	5·92	2·35	0·12	3·45	59·4
3	6·86	2·08	0·21	4·57	68·7
4	6·42	2·64	0·12	3·66	58·0
5	5·59	2·77	0·21	2·61	48·5
Mean	6·00	2·35	0·17	3·47	59·2

N balance and partition of urinary N

Table 3 shows the N balance results for the individual lambs. Lambs nos. 1–3 were given a smaller amount of N in the post-starvation period than in the pre-starvation period, owing to the reduced intake of milk in the first three feeds after starvation. Nevertheless, the urinary N was in all lambs much higher in the post-starvation than in the prestarvation period. The differences in urinary N excretion were reflected in a marked decrease in the percentage of the absorbed N which was retained. Faecal N was reduced slightly but not significantly during starvation. The urinary loss of N during starvation (mean for 2nd, 3rd and 4th days) was 478 mg N/kg live weight 24 h,

Table 4. *Partition of the urinary nitrogen* (*mean values and ranges for five lambs*)

	A Pre-starvation (6 days) (mg/24 h)	B Starvation (4 days) (mg/24 h)	C Post-starvation (6 days) (mg/24 h)	Significance of differences		
Component				A–B	B–C	A–C
Ammonia	46 (35–66)	198 (120–264)	87 (42–197)	***	*	NS
Urea	1282 (1207–1473)	1815 (1425–2247)	1778 (1542–2140)	**	NS	***
Amino acid	21 (18–29)	58 (44–74)	9 (0–45)	***	**	NS
Creatine	14 (10–18)	88 (27–109)	27 (15–59)	**	**	NS
Creatinine	71 (67–77)	79 (67–87)	70 (62–74)	NS	NS	NS
Uric acid	21 (19–23)	30 (25–37)	21 (16–26)	**	*	NS
Allantoin	43 (35–51)	115 (54–147)	37 (24–81)	**	**	NS
Purine base	40 (36–46)	109 (98–129)	56 (40–108)	***	**	NS
Residual	276 (168–362)	367 (259–653)	272 (184–339)	—	—	—
Total	1814 (1721–1924)	2845 (2096–3310)	2357 (2035–3003)	**	NS	**

NS, not significant. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.
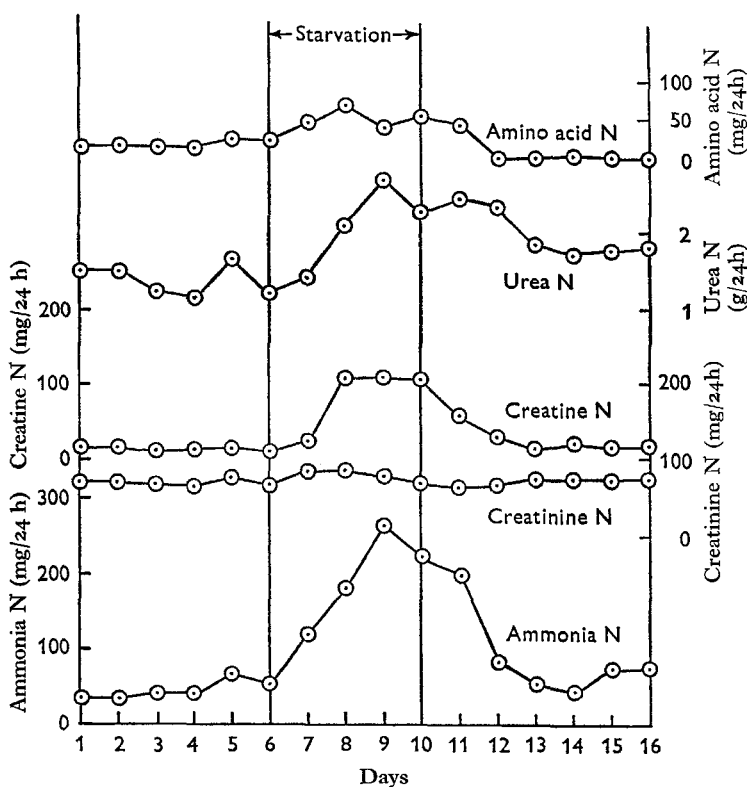

Fig. 1. Effect of starvation of the lambs on the partition of nitrogen
in their urine. Each value is the mean for five lambs.

compared with a value for the calf of 259 mg N (Blaxter & Wood, 1951) and 152 mg N
for the sheep (Morris & Ray, 1939). The high value for the lamb reflects the intensity
of N metabolism of the small growing animal.

The partition of the urinary N is shown in Table 4. The weights of all the consti-

tuents, with the exception of creatinine, increased during starvation and with the exception of urea and creatinine, subsequently decreased when food was reintroduced. Urea excretion remained high for 2 days after refeeding and then declined, but did not return to the normal levels of the pre-starvation period. This result is in agreement with those for the N balance and shows that the high excretion of urinary N in the post-starvation period was largely due to a high excretion of urea. The high urinary N excretion after starvation was also observed by Hutchinson & Morris (1936) with adult cows, and by Blaxter & Wood (1951) with milk-fed calves. These authors suggested
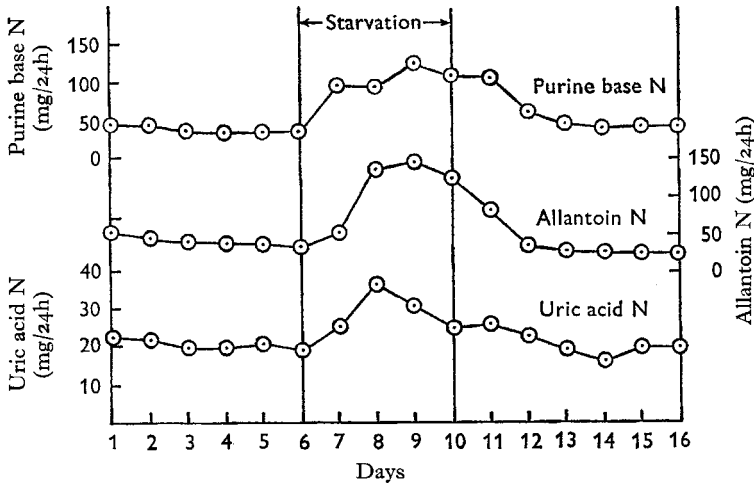

Fig. 2. Effect of starvation of the lambs on the partition of total purine nitrogen. Each value is the mean for five lambs.

that energy requirements may be higher than normal after starvation, resulting in the deamination of amino acids for energy purposes. Meyer & Clawson (1964) have shown that adult rats and sheep make greater weight gains after starvation, that they utilize the absorbed energy more efficiently, and that their gain in weight contains a higher proportion of fat and a lower content of protein than that made before starvation. The daily pattern of excretion of the individual urinary N constituents is shown in Figs. 1 and 2. Creatinine excretion remained constant during starvation, in agreement with Folin's (1905) concept of an endogenous creatinine excretion which is 'a constant quantity different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated'. Creatine excretion increased during starvation, owing to the breakdown of protoplasmic material. Ammonia excretion increased during starvation in accord with the normal effects of starvation in man in whom the breakdown of body fat results in ketosis, a high titratable acidity in the urine and a high excretion of ammonia. Total purine N excretion increased during starvation, suggesting that in addition to cytoplasmic protein, nucleoproteins were being broken down. In man there is little change in the weight of total purine N excreted during starvation, though daily fluctuations in the excretion of uric acid and purine base occur, and total purine N expressed as a

percentage of total urine N increases as the fast proceeds (Lusk, 1917). In the adult sheep, Morris & Ray (1939) found a decrease in the amount of the total N excreted as purine base metabolites, though the purine bases themselves increased both in amount and as a percentage of total N. In the lamb, purine bases and their metabolites increased during starvation both in amount and as a percentage of total N. The weights of purine base N and allantoin N increased by some 300% and of uric acid N by about

Table 5. *Partition of total purine nitrogen before and during starvation of the lamb, calf and sheep (expressed as a percentage of total purine N)*

Component	Lamb*		Calf†		Sheep‡	
	Before	During	Before	During	Before	During
Uric acid	20	11	4	7	5	8
Allantoin	41	49	74	71	83	62
Purine base	39	40	22	22	11	30
Total purine N (mg/24 h)	103	280	760	747	—	—

* This work.   † Blaxter & Wood (1951).   ‡ Morris & Ray (1939).

Table 6. *Partition of urinary nitrogen during starvation in the lamb, calf and sheep (expressed as a percentage of total urinary N)*

Component	Lamb*	Calf†	Sheep‡
Ammonia	8·3	7·6	3·9
Urea	72·2	72·5	57·3
Ammonia + urea	80·5	80·1	61·2
Amino acid	2·2	—	0·6
Creatine	4·0	3·1	4·9
Creatinine	2·9	3·7	7·9
Uric acid	1·1	0·6	1·4
Allantoin	5·0	5·9	18·4
Purine base	4·2	1·8	4·7
Total purine	10·3	8·3	24·5

* This work; values for 2nd, 3rd and 4th days of starvation.
† Table 11, Blaxter & Wood (1951).
‡ Recalculated from the results of Morris & Ray (1939). Mean for four sheep on 4th and 5th days of starvation.

50%. In Table 5 the percentage distribution of the purine N in the lamb is compared with that of the calf and the sheep. Blaxter & Wood (1951) observed no increase in the total purine N excretion of the calf during starvation and only small changes in the proportions of the individual constituents. In the sheep, Morris & Ray (1939) found considerable changes in the purine N distribution as a result of starvation. Whereas the proportion of purine base N remained constant in the lamb, there was an increase in the proportion of allantoin N and a decrease in the proportion of uric acid N.

The residual N was high in all periods and accounted for 10–15% of the total urinary N. No protein was detectable in the urine, and it must be assumed that undetermined nitrogenous constituents, and errors in the eight analytical methods, contributing to the total N, were responsible for this residual fraction.

The partition of the urinary N during starvation in the lamb is compared in Table 6

with that in the calf and the adult sheep. Whereas the results for the lamb and the calf are in close agreement, the sum of ammonia and urea represents a much higher percentage of the total N in the lamb than in the sheep. As noted above, the weight of creatinine excreted was unaffected by starvation, though as a percentage of total N the creatinine N decreased significantly during starvation from 3·9 to 2·9%. This value during starvation is lower than the corresponding value of 7·9% for the adult sheep (cf. Table 6) and is in agreement with the difference between the calf and the adult cow observed by Blaxter & Wood (1951). Creatinine N excretion in the calf represented a much lower percentage of the total urinary N during starvation (3·7%) than in the adult cow (7·3%). It would seem that the young animal of both species breaks down a greater proportion of its body protein during starvation than the adult, and this is reflected by the higher proportions of urea and ammonia, and the lower proportions of creatinine N in the urine.

Table 7. *A comparison between the endogenous nitrogen and sulphur loss in lambs given a N-free diet with the urinary N and S excretion during starvation*

Component	A Endogenous loss* (mg/kg live weight 24 h)	B Excretion during starvation† (mg/kg live weight 24 h)	Difference (B − A)
Nitrogen:			
Ammonia	14·0	34·6	+20·6
Urea	70·2	300·6	+230·4
Amino acid	3·3	9·0	+5·7
Creatine	2·3	16·7	+14·4
Creatinine	9·1	12·0	+2·9
Uric acid	2·9	4·8	+1·9
Allantoin	8·3	21·0	+12·7
Purine base	4·3	17·5	+13·2
Total	114·4	416·2	+301·8
Sulphur:			
Inorganic	0·4	13·4	+13·0
Ethereal	0·9	1·1	+0·2
Neutral	3·9	3·7	−0·2
Total	5·2	18·2	+13·0

* Mean values for six lambs; recalculated from Walker & Faichney (1964*b*).
† Mean values for five lambs for N components and for two lambs for S components; 2nd, 3rd and 4th days of starvation.

Table 8. *A comparison in the lamb between the contents of protein nitrogen and creatine N in muscle and in the urinary N excreted during starvation in excess of the endogenous N loss*

Constituent	Content in muscle		Content in excess urinary N (mg/kg live weight)
	(%)	In 9·93 g (mg)	
Protein N	2·900	288·0	284·5*
Creatine N	0·103	10·2	14·4
Total N	3·040	301·8	301·8

* Sum of ammonia N, urea N, amino acid N, and total purine N.

The urinary N excreted during starvation contains an endogenous N component. Table 7 compares the partition of the urinary N during starvation with the endogenous N excretion of lambs given a N-free diet (Walker & Faichney, 1964b). On the assumption that the excess N during starvation represents N derived from muscle breakdown it may be calculated that the breakdown of 9·93 g muscle/kg live weight per day would account for the excess N in the urine. A comparison between the composition of muscle and the nature of the excess N in the urine is shown in Table 8. Though the urinary excretion of creatine was greater than that expected from muscle katabolism, the protein N breakdown was almost wholly accounted for by the sum of the urea, ammonia, amino acid, and purine N excreted.

S excretion in the urine

Mean values for the partition of S in the urine for two of the five lambs in this experiment are shown in Table 9. As a result of analytical difficulties in the estimation of total S in the urine of the other lambs, their values were excluded. The daily excretion pattern of the S fractions is shown in Fig. 3. The increase in the excretion of total S during starvation was due almost entirely to an increase in the inorganic sulphate fraction. Ethereal sulphate excretion decreased slightly during starvation. The neutral S fraction did not remain constant, as was observed in man by Folin (1905), but rose during the post-starvation period. Folin commented that the excretion of neutral S resembled that of creatinine but was not nearly so constant. Folin attributed these fluctuations to a lack of accuracy in the analytical techniques. Since Folin's methods of analysis were used in the present experiment the same explanation might be applicable.

Table 9. *Partition of urinary sulphur (mg/24 h) in the lamb*
(mean values and ranges for two lambs)

Component	Pre-starvation	Starvation	Post-starvation
Inorganic S	22 (13–30)	69 (25–98)	52 (23–120)
Ethereal S	16 (13–19)	9 (4–14)	13 (9–17)
Neutral S	12 (10–16)	26 (16–33)	30 (25–34)
Total S	50 (43–59)	104 (72–127)	95 (70–161)

Table 10. *Mean values (mg/24 h) for the sulphur balance of two lambs*

Period	S intake*	Faecal S	Urinary S	S balance
Pre-starvation	362	25	50	+287
Starvation	—	11	106	−117
Post-starvation	362	20	95	+247

* S content of dried whole milk 2·69 mg/g dry matter.

Mean values for the S balance of lambs nos. 4 and 5 are shown in Table 10. The retention of S in the post-starvation period was less than in the pre-starvation period owing to an increased loss of urinary S. It is clear that this increased excretion was due mainly to the carryover effect from the starvation period, and that by the end of the recovery period the urinary excretion had returned to the level observed before starvation (cf. Fig. 3).

The ratio of N to S in the urine of man during starvation varies between 14 and 17 (Lusk, 1917) and in the adult ruminant it varies between species. Morris & Ray (1939) reported values between 10 and 17 for the goat, about 14 for the cow, and between 21 and 34 for the sheep on the 6th day of starvation. In the lamb, the ratio decreased from a mean value of 44 before starvation to individual daily values between 21 and 36 during starvation. These ratios for the lamb and adult sheep are in good agreement, but are much higher than would be expected if muscle protein was the source of both the N
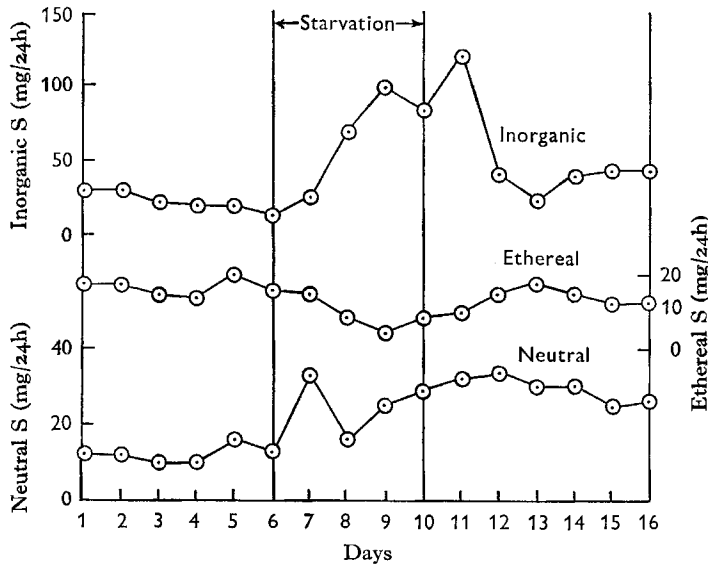
Fig. 3. Effect of starvation of lambs nos. 4 and 5 on the partition of sulphur in their urine.

and S in the urine. Analyses of muscle from a number of species show N to S ratios between 13 and 16 (Masters & McCance, 1939). During starvation the total N and S excreted in the urine contains an endogenous fraction as well as that derived from muscle katabolism. It might be expected that the net excretion of N and S, after subtracting the endogenous values (either total S or the neutral S fraction only) from the total excretion, would result in a N:S ratio similar to that of muscle. In fact, the mean ratio was not greatly affected, a value of 27 (or 24 if the endogenous neutral S alone was subtracted) being obtained (cf. Table 7). Walker & Cook (1967) showed earlier that wool growth in the lamb continues even under adverse nutritional conditions, and Marston (1948) demonstrated continued wool growth during total starvation in the adult sheep. It seems probable that, as a result of the demand for S for wool growth, S derived from tissue katabolism during starvation is retained by the body and less is excreted in the urine. The comparison shown in Table 14, of the minerals lost from muscle with those excreted in urine, supports the hypothesis that less S was being excreted than would be expected from the weight of muscle broken down. The observed ratio of N to S in lamb muscle was 16·2.

Table 11. *Titratable acidity of the urine and the excretion of ketone bodies*
by the lambs (mean values and ranges for five lambs)

Component	Pre-starvation	Starvation	Post-starvation
Titratable acidity (ml 0·1 N-NaOH/24 h)	33·9 (29·5–38·6)	131·2 (46·3–174·8)	39·1 (13·3–136·1)
Ketone bodies, expressed as acetone (mg/24 h)	78 (55–104)	508 (93–993)	230 (64–811)

Table 12. *Urinary excretion (mg/24 h) of minerals by the lambs*
(mean values and ranges for five lambs)

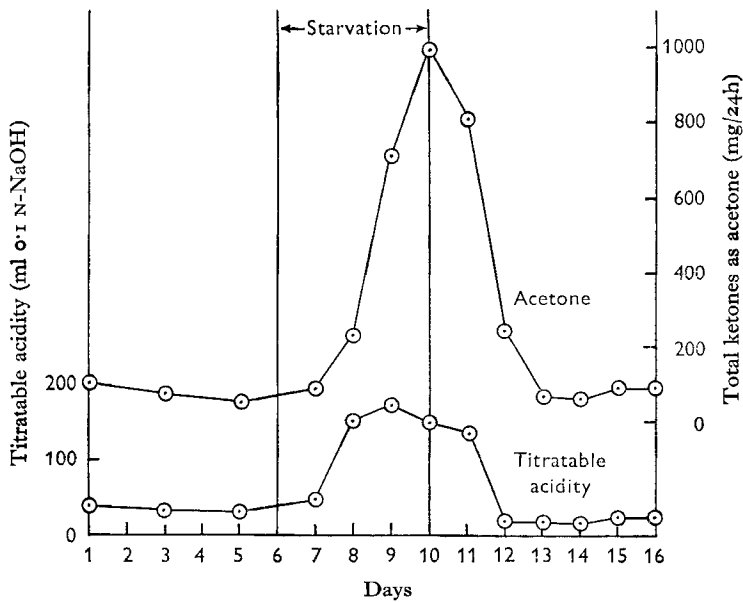| Element	Pre-starvation	Starvation	Post-starvation
Cl	1073 (960–1169)	580 (373–1094)	849 (329–1181)
P	190 (168–226)	428 (368–488)	193 (135–319)
Na	365 (312–422)	239 (131–505)	314 (69–473)
K	1087 (1042–1148)	644 (441–992)	1138 (547–1478)
Mg	51 (45–55)	43 (37–46)	45 (41–53)
Ca	4 (3–4)	7 (6–9)	7 (7–9)

Fig. 4. Effect of starvation of the lambs on titratable acidity and excretion of acetone in their urine. Each value is the mean for five lambs.

Titratable acidity and the excretion of acetone in the urine

An increased excretion of ammonia occurs during starvation in man, not only to neutralize the acids excreted owing to ketone acidosis but also to spare the loss of alkali from the reserves of buffer salts in the body (Peters & Van Slyke, 1946). As noted previously, this pattern of increased ammonia excretion was observed in all
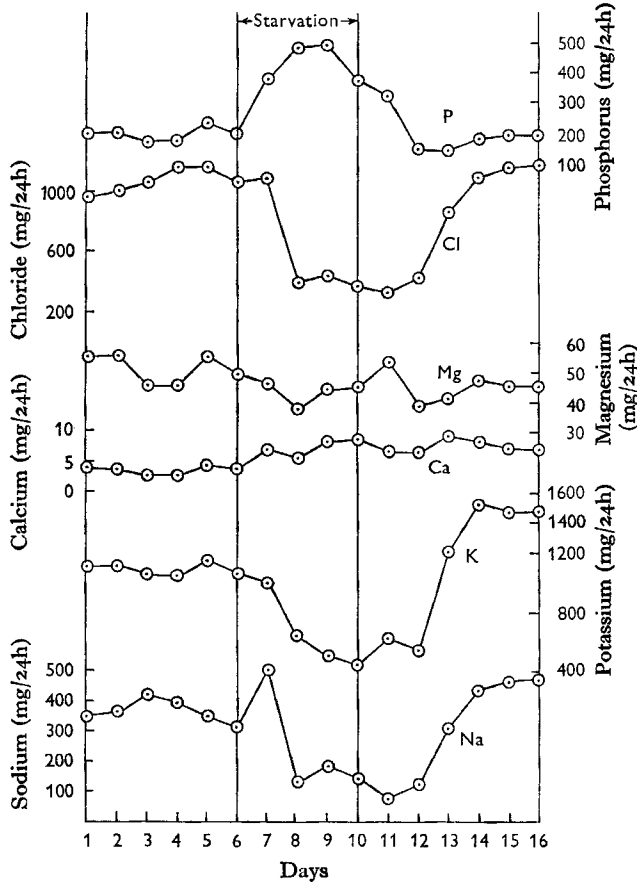

Fig. 5. Effect of starvation of the lambs on mineral excretion in their urine. Each value is the mean for five lambs.

lambs during starvation. Table 11 gives the mean values for the titratable acidity and excretion of ketone bodies, expressed as acetone, in all periods. The daily patterns of titratable acidity and acetone excretion are shown in Fig. 4. There was a delayed rise in both the titratable acidity and excretion of ketone bodies at the onset of starvation which continued to a peak at the end of starvation and gradually returned to normal levels on refeeding.

Mineral excretion in the urine

Mean values for the excretion of minerals in the urine are shown in Table 12 and the daily pattern is shown for each mineral in Fig. 5. There was a marked reduction in the excretion of Cl, K and Na during starvation, an increase in the excretion of P, but little change in the excretion of Ca and Mg.

The most obvious source of the minerals excreted in the urine during starvation is from the breakdown of body tissue. Most workers who have studied total starvation have calculated the ratio between the amounts of the different minerals lost during starvation and the loss of N, and related this ratio to that in muscle tissue. In many instances after prolonged starvation the ratio for certain minerals was similar to the calculated value, though frequent discrepancies occurred (cf. Gamble, Ross & Tisdall, 1923; Lusk, 1917; Blaxter & Wood, 1951). Since the author was unable to find complete analyses of lamb muscle at various ages, a number of muscle samples were

Table 13. *Mean values (per 100 g fresh muscle) for the composition of skeletal muscle of three lambs aged 4 weeks*

Dry matter (g)	22·45	K (mg)	427
Total N (g)	3·04	Na (mg)	81
Total S (mg)	188	Cl (mg)	44
P (mg)	149	Ca (mg)	7
		Mg (mg)	33

Table 14. *Comparison of the calculated mineral content of muscle of five lambs with the urinary loss of minerals on the 2nd, 3rd and 4th days of starvation*

Element	Amount in muscle when 528 mg K present (mg)	Mean daily excretion in urine during starvation (mg)
K	528	528
Na	100	151
Cl	53	409
Ca	9	7
Mg	41	42
P	184	444
S	232	114

analysed. The muscle samples were obtained from the thigh region of three lambs aged 4 weeks at slaughter. The results of these analyses are shown in Table 13. The lambs were bled at slaughter, though it was realized that this alone may have resulted in low values for certain minerals, particularly Na and Cl, owing to the loss of extra-cellular fluid (Widdowson & Southgate, 1959). Dickerson & Widdowson (1960) have also shown that the mineral composition of skeletal muscle in the young pig changes rapidly during early life, so that the values determined on lambs aged 4 weeks may not be applicable to other age-groups. Nevertheless, the values obtained are preferable to those for another species and are in reasonable agreement with published values for mammalian muscle.

On the assumption that all the K excreted in the urine during starvation was derived from muscle katabolism, and ignoring a possible endogenous K content, a

comparison is shown in Table 14 of the mineral content of the muscle katabolized during the 2nd, 3rd and 4th days of starvation with the quantities excreted on these days. It is apparent from this comparison that the quantities of Ca and Mg excreted could have arisen from the calculated quantity of muscle destroyed. All the other minerals were present in the urine in quantities in excess of those expected from muscle breakdown alone. The amounts of S excreted were less than expected on the basis of the muscle composition, whilst the excretion of Na, Cl and P was considerably in excess.

The N to P ratio in the urine of man during starvation is about 23 (Lusk, 1917) and for adult sheep it varies from 15 to 29 on the 6th day of starvation (Morris & Ray, 1939). In the lamb, ratios between 4 and 11 were observed during starvation compared with a N to P ratio in lamb muscle of 20 (cf. Table 13). The excretion of P in the urine was in excess of that derived from muscle breakdown alone (cf. Table 14) and would thus account for the low N to P ratios observed. Widdowson (1959) has reported extremely high values for the inorganic P content of the extracellular fluid in the newborn child. Since there is little information for the lamb at a comparable age, it seems possible that the P content of the extracellular fluid may also be high in early life. There was no evidence from the amounts of Ca excreted in the urine that extensive breakdown of bone had occurred during starvation.

Table 15. *Pulse rate, respiratory rate and rectal temperature of the lambs (mean values and ranges for five lambs)*

	Pre-starvation	Starvation	Post-starvation
Pulse rate (beats/min)	141 (130–150)	97 (77–113)	105 (85–115)
Respiratory rate (respirations/min)	37 (30–44)	21 (14–25)	21 (17–25)
Rectal temperature (°F)	102·7 (102·4–103·1)	100·7 (99·8–101·3)	102·3 (101·6–102·6)

Pulse rate, respiratory rate, rectal temperature and heat production

The mean values for the pulse rate, respiratory rate and rectal temperature of the lambs in each period are given in Table 15 and the daily pattern is shown in Fig. 6. Starvation caused a fall in all three measurements, but whereas the rectal temperature returned to the pre-starvation level on refeeding after starvation, both respiratory rate and pulse rate became constant at a lower level. The higher values before starvation may have been caused by the lack of training of the lambs, though they were all accustomed to handling and were allowed several minutes to settle quietly before measurements were taken.

Table 16 gives the individual values for the two lambs whose heat production was measured during the starvation period. Lamb no. 1 showed a decline in heat production from the 1st to the 2nd day of starvation, but the values on the 2nd, 3rd and 4th days showed no further decline. The measurements of the RQ with this lamb were not fully reliable owing to the short period of collection of the exhaled CO_2. Lamb no. 4 was kept in the respiration chamber for 6 h on both the 2nd and 3rd days of starvation

Table 16. *Heat production of lambs during starvation*

Lamb no.	Time after last feed (h)	Live weight (kg)	Surface area* (m²)	Temperature		RQ	Oxygen con- sumed (l./h)	Heat production		
				Rectal (°F)	Chamber (°F)			kcal/ h	kcal/ kg h	kcal/ m² h
I	3	5·5	0·331	103·6	86·9	0·70	3·62	17·0	3·08	51·4
	6	5·5	0·331	103·6	91·8	0·88	3·32	16·3	2·95	49·2
	8	5·5	0·331	104·0	78·6	0·74	3·63	17·2	3·12	52·0
	27	5·3	0·324	102·2	83·5	0·69	2·88	13·5	2·54	41·7
	30	5·3	0·324	103·8	87·8	0·94	2·81	14·0	2·63	43·2
	51	5·1	0·317	102·6	87·4	0·83	3·02	14·6	2·85	46·1
	80	5·0	0·311	103·3	86·0	0·82	2·69	13·0	2·62	41·8
4	27	7·0	0·381	103·8	87·6	0·76	2·92	13·9	1·99	36·5
	50	6·7	0·372	101·8	84·2	0·74	2·75	13·0	1·94	34·9
Mean for six lambs†	36	5·3	0·324	103·5	86·9	0·75	3·17	15·0	2·82	46·3

* Calculated from the equation of Peirce (1934): (m^2 area) = 0·121 (kg body-weight)$^{0·59}$.
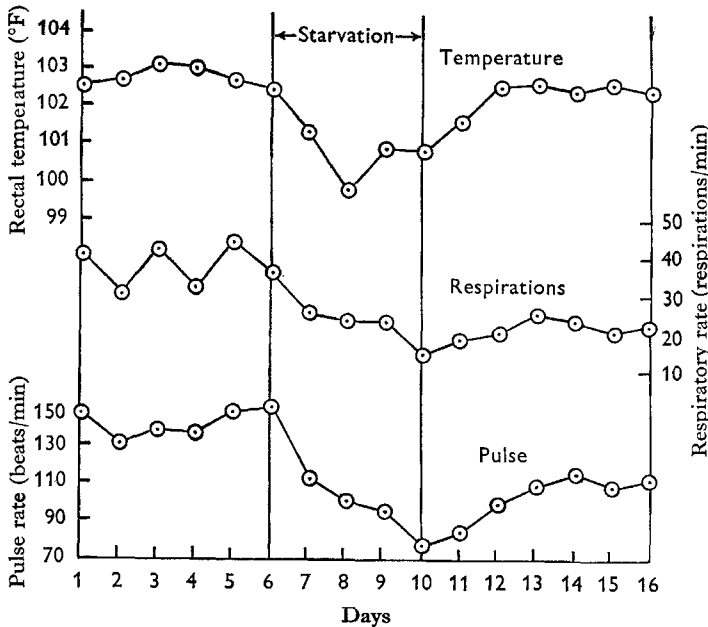† Walker & Faichney (1964*a*).

Fig. 6. Effect of starvation of the lambs on their rectal temperature, respiratory rate and pulse rate. Each value is the mean for five lambs.

to allow more accurate measurement of the RQ. The decline in heat production of this lamb was very small from the 2nd to the 3rd day of starvation.

These two lambs were aged about 3 weeks at the beginning of the starvation period, and their metabolic rates were in close agreement with those of the starved newborn lamb (Alexander, 1962). During the 1st day of starvation the heat production of lamb no. 1 some 5 h after feeding was about 50 kcal/m² h, compared with a value between 50 and 60 kcal/m² h for the newborn lamb 5 h after birth. At 48 h after

feeding or birth the comparable values were 35–45 kcal/m² h for lambs nos. 1 and 4, and 40 kcal/m² h for the newborn lamb. These values are also in good agreement with those obtained previously with six lambs, aged about 5 weeks, which were starved for 36 h before measurement of their metabolic rates. The comparable value was 46 kcal/m² h (Walker & Faichney, 1964*a*).

Blood urea concentration during starvation

Alexander (1962) measured the concentration of urea in the blood of newborn lambs during starvation, and found that the values increased from 4–20 mg urea N/100 ml at birth to 80–120 mg/100 ml 80–120 h after birth. The mean rate of increase was approximately 1 mg/h of starvation, and did not appear to be affected by prenatal nutrition or environmental temperature. Lambs given water or physiological saline during starvation had the same increase in blood urea concentration as lambs from which water was withheld. In the present experiment samples of blood were taken twice daily from three lambs during starvation. Since no change in blood urea concentration was observed, two further lambs were starved and water was withheld as well as food. Again there was no increase in blood urea concentrations. The results of this experiment are given in Table 17.

Table 17. *Mean values (mg urea N/100 ml) for the blood urea concentration of three lambs during starvation*

Treatment during starvation	Pre-starvation am	Starvation							
		Day 1		Day 2		Day 3		Day 4	
		pm	am	pm	am	pm	am	pm	am
Water given	7·8	9·1	9·2	10·2	11·3	11·3	9·1	9·4	11·5
Water withheld	9·3	10·2	11·9	11·5	12·6	—	—	—	—

It is apparent that the rise in blood urea concentration observed by Alexander (1962) was an effect of the age of the lamb and was due to the relative immaturity of the kidney in its ability to excrete urea. This situation is analogous to that discussed by McCance & Widdowson (1964) for a variety of other young animals. At 3 weeks of age it was clear that the lamb kidney had matured to the stage at which urea from endogenous or exogenous sources could be readily excreted.

DISCUSSION

The lambs in this experiment were able to withstand a starvation period of 4 days without noticeable distress, though their loss of body-weight represented some 15% of their pre-starvation live weight. Alexander (1962) found that when newborn lambs, born from ewes fed at a high plane of nutrition during pregnancy, were starved to death at 23° in still air, they survived for almost 3 days but lost about 25% of their body-weight before death. The provision of water during starvation did not prolong the survival time. Alexander concluded that death was due to the exhaustion of energy reserves, even though 'structural' fat was still present to the extent of 1·25% of the

weight at death. At birth these lambs had about 3% of fat in the body, so that the rate of fat depletion was about 1 g/h throughout the starvation period. The lambs in the present experiment were heavier and older than the lambs used in Alexander's studies, and were estimated to have a fat content of 4·5% before starvation. This estimate was based on the calculated empty live weight of the lambs, and on the close correlation which has been found between the weight of fat in the body and the empty live weight of lambs which are growing on a diet of reconstituted dried whole milk (unpublished results).

Table 18. *Calculated values for the depletion of the body reserves of fat in the lamb after 96 h starvation*

Live weight before starvation (kg)	6·50
Empty live weight before starvation (kg)	6·05
Fat in empty body before starvation (%)	4·50
Weight of fat in body before starvation (g)	272
Loss of fat during starvation (g/h)*	1·04
Loss of fat in 96 h (g)	100
Weight of fat in body at end of starvation (g)	172
Loss in body-weight in 96 h (kg)	1·00
Fat in empty body at end of starvation (%)	3·40

* Calculated from the results of Alexander (1962) for the loss in fat during starvation of newborn lambs.

Table 19. *Calculated partition of body-weight loss (g/24 h) of lamb no. 1 on the 2nd and 3rd days of starvation*

Total body-weight loss	274
Fat	23
Protein	18
Carbohydrate	3
Extracellular water	67
Intracellular water	130
Loss accounted for	241

On the assumption that the lambs contained 4·5% fat in the empty body before starvation, and lost fat at the same rate as the newborn lamb, the calculation in Table 18 shows that after 4 days starvation the fat content of the lamb would still be about 3·4%. The loss of fat of 1 g/h during the starvation of newborn lambs (Alexander, 1962) is in agreement with the calculated loss of 0·96 g/h on the 2nd and 3rd days of starvation (cf. Table 19). These calculations are clearly only approximations, but they suggest that a starvation period of 4 days does not impose a severe burden on a lamb aged 3 weeks, provided that it has been well fed from birth and has been able to increase its reserves of body fat.

The loss in body-weight during starvation is composed of carbohydrate, fat, protein and water, though the proportions of the energy constituents lost at any particular stage of starvation will depend upon the initial reserves and the precise time within the starvation period at which a calculation is made. An estimate of the protein loss is given by the urinary N excretion, and the non-protein RQ may be used to estimate the loss of fat and carbohydrate. In this experiment the RQ was determined on one lamb on both the 2nd and 3rd days of starvation. The procedure used by Dewar &

Newton (1948–9) was applied to the calorimetric results, and the calculated composition of the body-weight loss for lamb no. 1 is given in Table 19. Comparison of this result with those of Alexander (1962) for the newborn lamb shows that, although the protein loss was greater and the carbohydrate loss much lower, the loss of fat was similar. Since the measurements in the present experiment were made on the 2nd and 3rd days of starvation it would be expected that the carbohydrate reserves would be largely exhausted at this stage, and that fat and protein would comprise the major sources of energy.

The extracellular and intracellular water losses were calculated from the quantities of Na and K excreted in the urine during starvation by the method of Gamble *et al.* (1923). The composition of the intracellular water was taken from the analysis of lamb muscle (cf. Table 13), and that of the extracellular water from the analysis of sheep plasma by Kaiser & Cummings (1958). It is apparent from this calculation that the intracellular water contributes the major portion of the body-weight loss.

After starvation, the daily gain in weight of the lambs was greater than that observed during the pre-starvation period, even though the N retention was less. This suggests that the composition of the gain in weight was very different after starvation, and that the gain probably contained more water and fat and less protein than was present in the gain before starvation. Meyer & Clawson (1964) have reported differences in the composition of the gain before and after partial starvation in both rats and sheep, and a more rapid or compensatory growth.

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