Nitrogen balance studies with the milk-fed lamb

2.* The partition of urinary nitrogen and sulphur

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The studies of Folin (1905*a*) on the composition of normal human urine led him to propose (Folin, 1905*b*) that certain nitrogen and sulphur constituents in the urine are endogenous, and are excreted as a fixed proportion of live weight. The amounts of other constituents vary with the composition of the diet and are diminished in quantity when diets low in N and S are given.

In an earlier experiment (Walker & Faichney, 1964) six lambs were given N-free diets and the urine and faeces were collected quantitatively. The urine of these lambs was analysed and the results are presented in this paper. Since to our knowledge the urine of the milk-fed lamb has not previously been analysed for its N- and S-containing constituents, these analyses are discussed with reference to the laws of Folin and are compared with the urine composition of other animals.

EXPERIMENTAL

Total N was estimated daily in the 24 h yield of urine from each lamb. The urine was then bulked for 2 or 3 days. The diet and treatment of the lambs and the methods for the collection and preservation of the urine are given in the previous paper (Walker & Faichney, 1964). Table 5 of that paper shows the samples that were used for the detailed analysis of the urine. The analytical results in the present paper are shown as a single value representing each period, but the estimations were made on two or three bulk samples within each period.

Analytical methods

Diet and faeces. Total S was determined by the method of Benedict (1909), the reagent of Denis (1910) being used.

Urine. Total N was determined by the Kjeldahl method, ammonia and urea by the method of Conway (1957), amino acids and uric acid by the method of King & Wootton (1956), purine bases by the method of Hitchings & Fiske (1941), creatine and creatinine by the method of Folin (1914), allantoin by the method of Christman, Foster & Esterer (1944), total S by the method of Benedict (1909) with the reagent of Denis (1910), total sulphate, inorganic sulphate, ethereal sulphate and neutral S by the method of Folin (1905–6) and reducing sugar by the method of Shaffer & Hartmann (1920–1).

* Paper no. 1: *Brit. J. Nutr.* (1964), 18, 187.

RESULTS

Partition of N and S

Table 1 shows the partition of N and S in the urine of individual lambs and the mean values for each period. The values for lambs nos. 108 and 109, given the low-protein diet (no. 6) in the preliminary period, are excluded from the means for that period. Individual mean live weights, calculated from the regression analyses of the daily weights, are also given in Table 1. A comparison of the means for each period, whether expressed as total excretion (mg/24 h) or related to live weight (mg/kg 24 h),

Table 1. *Partition of nitrogen and sulphur in the urine (mg/24 h), and mean live weight (kg) of individual lambs in each period of the experiment*

	Preliminary period, lamb no.						
	101	106	107	108*	109*	111	Mean
Live weight...	5·31	5·14	5·17	4·17	4·91	5·29	5·00
Nitrogen:							
Ammonia	72	86	37	42	68	107	76
Urea	1253	1443	1780	613	743	1695	1543
Amino acid	20	16	6	2	13	15	14
Creatine	54	28	54	20	24	43	45
Creatinine	45	54	52	36	45	57	52
Uric acid	15	14	30	17	12	30	22
Allantoin	32	41	51	39	47	60	46
Purine base	25	18	19	20	26	29	23
Residual	285	156	563	178	143	405	352
Total	1801	1856	2592	967	1121	2441	2173
Sulphur:							
Inorganic	21	20	30	5	7	32	26
Ethereal	14	8	20	15	11	9	13
Neutral	13	14	38	10	13	11	19
Total	48	42	88	30	31	52	58
	N-free period, lamb no.						
	101	106	107	108	109	111	Mean
Live weight...	5·71	5·48	5·30	4·16	5·01	5·28	5·16
Nitrogen:							
Ammonia	70	97	28	43	78	116	72
Urea	375	233	508	297	277	481	362
Amino acid	49	17	2	0	29	2	17
Creatine	4	4	14	6	16	29	12
Creatinine	47	51	53	37	42	54	47
Uric acid	14	6	19	17	13	22	15
Allantoin	56	40	39	27	38	60	43
Purine base	26	21	16	16	23	29	22
Residual	127	47	22	11	76	75	60
Total	768	516	701	454	592	868	650
Sulphur:							
Inorganic	7	1	0	1	1	0	2
Ethereal	11	3	6	3	5	1	5
Neutral	24	12	23	12	29	22	20
Total	46	16	29	16	35	23	27

Table 1 *cont.*

	Recovery period, lamb no.						
	101	106	107	108	109	111	Mean
Live weight...	5·69	5·42	5·57	4·25	5·04	5·38	5·23
Nitrogen:							
Ammonia	130	77	76	61	82	122	91
Urea	1392	1273	1826	1118	1117	1476	1367
Amino acid	64	18	2	10	15	0	18
Creatine	54	39	74	55	52	81	59
Creatinine	51	63	65	34	39	52	49
Uric acid	7	4	22	12	12	21	13
Allantoin	32	36	32	33	33	36	34
Purine base	21	28	20	25	23	22	23
Residual	136	193	183	57	175	73	137
Total	1887	1731	2291	1405	1548	1883	1791
Sulphur:							
Inorganic	29	9	50	6	18	5	19
Ethereal	16	13	22	15	31	15	19
Neutral	14	16	7	9	14	14	12
Total	59	38	79	30	63	34	50

• Values for lambs nos. 108 and 109 were omitted from the mean values of the preliminary period.

showed that there was a significant decrease in the content of total N ($P < 0·001$), urea ($P < 0·001$), creatine ($P < 0·01$), total S ($P < 0·05$), inorganic sulphate ($P < 0·05$) and ethereal sulphate ($P < 0·05$) in the N-free period, and a significant increase on changing back to the medium-protein diet (no. 3) in the recovery period. The weight of allantoin excreted (mg/24 h) was significantly lower ($P < 0·05$) in the recovery period than in either the preliminary or N-free periods, and that of neutral S was significantly lower ($P < 0·05$) in the recovery period than in the N-free period. All other differences were not significant. These results suggested that the quantity of certain constituents, such as ammonia, amino acids, creatinine, uric acid and purine bases, was independent of the diet. Blaxter & Wood (1951) found that calves given N-free diets excreted urine that contained over 2% of reducing sugar, a level which caused interference in the estimation of allantoin by the method of Larson (1931–2). The mean values for the reducing sugar excreted in each period of our experiment expressed as mg glucose/100 ml urine were:

Preliminary period	113 (range 69–147)
N-free period	84 (range 37–164)
Recovery period	158 (range 90–202)

The highest value observed did not exceed 0·2%.

DISCUSSION

The concepts of Folin

The results of Folin (1905*a*), on the chemical composition of human urine, have been taken as a standard to compare the urinary excretion of N and S in different species. It is of value to restate these findings and to discuss them with reference to our analyses of lamb's urine.

Folin's laws governing the chemical composition of urine stated: (1) that creatinine excretion is 'a constant quantity different for different individuals, but wholly independent of quantitative changes in the total amount of nitrogen eliminated'; (2) that 'when the total amount of protein-metabolism is greatly reduced, the absolute quantity of uric acid is diminished, but not nearly in proportion to the diminution in total nitrogen'; (3) that in circumstances of low N intake 'there is usually...a decrease in the absolute quantity of ammonia eliminated'; and finally (4) that urea is 'the only nitrogenous substance which suffers a relative as well as an absolute diminution with a diminution in the total protein-metabolism'. Folin also stated that the excretion of neutral S resembled that of creatinine, yet was 'not nearly so constant as that of the kreatinin', which he ascribed to a lack of accuracy of the analytical technique. This lack of constancy was noted by Amann (1933), who found that the excretion of neutral S was the same on low- as on 'normal'-protein diets, but was increased on high-protein diets. Among the other S constituents, Folin observed that the excretion of inorganic sulphate resembled that of urea and that the absolute quantity of ethereal sulphate diminished as the total excretion of S diminished, but to a much smaller extent. Excretion of ethereal sulphate was, therefore, analogous to that of uric acid and ammonia.

Our observations on lamb's urine agreed with those of Folin so far as urea, creatinine, inorganic sulphate, ethereal sulphate and neutral S were concerned, but no decrease in the excretion of uric acid and ammonia was observed. Excretion of other nitrogenous constituents not studied by Folin, such as allantoin, purine bases and the amino acids, showed no decrease as the excretion of total N decreased.

Table 2. *Creatinine nitrogen excretion of different animals, related to total nitrogen excretion and to basal heat production*

Species		$\frac{\text{Creatinine N}}{\text{Total N}} \times 100$	mg creatinine N/kcal basal heat production	Reference
Mouse	adult	4·92	0·095	Smuts (1935)
Rat	adult	7·11	0·141	Smuts (1935)
Rat	adult	11·00	—	Ashworth & Brody (1933)
Guinea-pig	adult	7·18	0·137	Smuts (1935)
Rabbit	adult	14·30	0·304	Smuts (1935)
Pig	adult	18·20	0·378	Smuts (1935)
Ox (calf)		11·80	0·232	Blaxter & Wood (1951)
Sheep (lamb)		7·50	0·131	Present paper; Walker & Faichney (1964)

Brody (1945) stated that, for growing animals and for animals of the same species but of different size, creatinine excretion varied directly with live weight. With milk-fed lambs in our experiment this value was between 9·17 and 9·95 mg creatinine N/kg live weight 24 h, which compares well with the value of 10·7, derived from the equation of Brody, Proctor & Ashworth (1934) for adults of different species, or the value of 6·6 for adult sheep (Hunter, 1914, 1915; Palladin, 1924; quoted by Brody, 1945).

The creatinine N excretion is frequently expressed as a percentage of the total endogenous N. Brody (1945) stated that for mature animals of different species this percentage increased with increasing live weight from 4% for animals weighing 20 g to 30% for those weighing 1000 kg. Some selected values for this percentage are given in Table 2. It has been suggested that when in a particular animal the percentage is at a maximum, then the animal has reached its minimum endogenous level of N excretion (Ashworth & Brody, 1933). Comparisons of this percentage among the different bulk urine samples taken from our lambs during the N-free period confirmed that the samples taken for N balance estimations (Walker & Faichney, 1964, Table 5) coincided with maximum value for the percentage.

Creatinine N excretion in mature animals has also been related to the basal heat production. Smuts (1935) found that there was an increase in creatinine excreted per unit basal heat production with an increase in the size of the animal (Table 2).

Table 3. *Sulphur balances (mg/24 h) of individual lambs in each period of the experiment*

Lamb no.	Intake	Faeces	Urine	Balance
		Preliminary period		
101	213·6	18·5	48·0	+147·1
106	200·0	38·2	42·0	+119·8
107	215·5	3·8	88·0	+123·7
108*	62·2	29·7	30·0	+2·5
109*	74·0	18·9	31·0	+24·1
111	223·2	33·1	52·0	+138·1
Mean*	213·1	23·4	57·5	+132·2
		N-free period		
101	0·8	30·5	46·0	−75·7
106	0·8	12·0	16·0	−27·2
107	0·8	24·5	29·0	−52·7
108	0·7	12·6	16·0	−27·9
109	0·8	36·3	35·0	−70·5
111	0·8	17·3	23·0	−39·5
Mean	0·8	22·2	27·5	−48·9
		Recovery period		
101	213·6	32·3	59·0	+122·3
106	200·0	46·4	38·0	+115·6
107	215·5	16·6	79·0	+119·9
108	175·6	32·9	30·0	+112·7
109	211·0	15·3	63·0	+132·7
111	221·2	46·7	34·0	+140·5
Mean	206·2	31·7	50·5	+124·0

* Values for lambs nos. 108 and 109 were omitted from the mean values of the preliminary period.

Apparently this generalization does not apply to immature animals, since in Table 2 the values for three calves (mean weight 31·7 kg) and for six lambs (mean weight 5·2 kg) do not fit into their correct position when compared with values for mature animals of these species.

Excretion of S

During the N-free period the intake of S was derived solely from the mineral supplement in solution and was considerably below the intake during either the preliminary or recovery periods. Four of the six lambs lost patches of wool after being given the N-free diet, the loss being noticed at between 7 and 16 days on this diet. The S balances of individual lambs, with mean values for the periods, are shown in Table 3. Values for lambs nos. 108 and 109 were excluded from the mean for the preliminary period, since their intake of S was lower when given diet no. 6 than that of lambs given diet no. 3. There was good agreement between the S balances of lambs given diet no. 3 in the preliminary and recovery periods. When the endogenous daily loss of S (mean 48·9 mg) was subtracted from the mean value for daily intake in the preliminary and recovery periods, the differences were 164·2 and 157·3 mg respectively. These calculated balances were in excess of the observed balances by 32·0 and 33·3 mg respectively; the differences were significant ($P < 0{\cdot}05$ and $P < 0{\cdot}01$). The reason for the increased excretion of S is not known, unless it is assumed that the intake was in excess of requirement.

A calculation which involved several assumptions showed that the positive balance of 130 mg was approximately the amount of S that would be required to meet the needs of a lamb of average weight 5·2 kg for wool production and live-weight gain (Table 4).

Table 4. *Calculation of the sulphur requirement of a lamb for wool growth and live-weight gain*

		Source of value
Live weight of lamb (kg)	5·2	Mean value, Table 1
Live-weight gain (g/24 h)	70	Mean value, Table 6 of Walker & Faichney (1964)
S content of the body (%)	0·15	Hogan & Nierman (1927)
S content of wool (%)	3·38	Corfield & Robson (1955)
Surface area of lamb (cm²)	3200	Peirce (1934)
Surface area producing wool (%)	85	B. F. Short*
Wool growth (mg/cm² 24 h)	0·6	B. F. Short*
Wool growth (mg/24 h lamb)	1572	—
S content of wool growth (mg/24 h lamb)	53	—
S content of live-weight gain† (corrected for weight of wool; mg/24 h lamb)	102	—
S requirement for wool growth and live-weight gain (mg/24 h lamb)	155	—

* Private communication from Dr B. F. Short, CSIRO, Prospect, NSW, Australia.
† On the assumption that greasy wool contains 75% by weight of clean wool.

The mean ratio of endogenous N to S in the urine produced in the N-free period was 27·8, which was not significantly lower than the values for the preliminary and recovery periods (36·2 and 37·6 respectively) for lambs fed on diet no. 3. Since the intake of S was very low in the N-free period, this ratio was probably characteristic of the endogenous level.

SUMMARY

1. The partition of nitrogen and sulphur in the urine of six male Merino lambs given medium-protein, low-protein and N-free diets is reported.

2. The weights of total N, urea, creatine, total S, inorganic and ethereal sulphates were lower during the N-free period than when the medium-protein diet was given.

3. The weights of ammonia, amino acids, creatinine, uric acid, allantoin and purine bases were not significantly altered when the lambs were given the N-free diet.

4. The weights of neutral S and of allantoin excreted were similar in the preliminary and N-free periods, but decreased when the lambs were given the medium-protein diet in the recovery period.

5. The S balances in the preliminary and recovery periods were similar (+130 mg/24 h). In the N-free period the intake of S was small (0·08 mg/24 h) and the S balance (−50 mg/24 h) could be considered endogenous.

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