Brit. J. Nutr. (1964), **18**, 187, *with 3 plates*

Nitrogen balance studies with the milk-fed lamb

1. Endogenous urinary nitrogen, metabolic faecal nitrogen and basal heat production

BY D. M. WALKER AND G. J. FAICHNEY
Department of Animal Husbandry, University of Sydney, Australia

(*Received* 20 *June* 1963—*Accepted* 3 *December* 1963)

The endogenous urinary nitrogen excretion of mature animals of several different species has been shown to be related to the basal heat production, the ratio being 2 mg N/basal kcal (Smuts, 1935).

The question of whether this ratio is applicable to the rapidly growing young animal has not been satisfactorily answered (Sorg-Matter, 1928; Ashworth, 1935; Ashworth & Cowgill, 1938; Treichler, 1939), though it has been shown that for the calf the ratio is similar to that found for adult cattle (Blaxter & Wood, 1951*a*).

The purpose of the experiment described here was to determine the endogenous urinary N excretion of the milk-fed lamb, as a necessary preliminary to the estimation of the protein requirements of the lamb by the factorial method (Mitchell, 1929; Blaxter & Mitchell, 1948), and to relate the endogenous N excretion to the basal heat production.

EXPERIMENTAL AND RESULTS

Experimental plan

The experiment was divided into four periods: (1) a preliminary period of 9–14 days when a medium-protein diet (lambs nos. 101, 106, 107 and 111) or a low-protein diet (lambs nos. 108 and 109) was given; (2) a period of 8–14 days when no N was given; (3) a recovery period of 5–10 days when the medium-protein diet was given; (4) a final period when the lambs were deprived of food but not of water, for 36 h, after which the basal heat production was measured.

Periods 2 and 3 were varied in length, their duration being dependent upon whether individual lambs would drink the N-free diet without refusals, and upon the daily pattern of N excretion in the urine. Table 1 gives the ages of the lambs at the beginning of the experiment and the length of each period.

Lambs

Six male Merino lambs, born at pasture, were taken away from the ewe when they were about 1 week old and were housed individually in metabolism cages. The animal house was uninsulated and the air temperature varied between 45 and 75° F over the period of the experiment.

Collection of faeces and urine

Three sizes of metabolism cages were used. Lambs weighing from 2 to 6 kg were housed in cages 66 cm long, 21 cm wide and 51 cm high; lambs weighing from 6 to 10 kg in cages 66 cm long, 25 cm wide and 61 cm high, and lambs weighing from 10 to 15 kg in cages 74 cm long, 30 cm wide and 68 cm high. The sides and back of each cage were made of galvanized iron sheet (0·7 mm thick). The back was made so that it could be swung open or removed for access to the cage. The front and top of each cage were made of a large-aperture wire screen (2 cm mesh size). The lambs were fed by bottle and nipple through the wire screen at the front of the cage. The floor of each cage consisted of strong wire mesh (diam. 2 mm; mesh size 1 cm). The cages were placed on a stand and a rectangular funnel slightly larger in area than the base of the cage and made of galvanized iron sheet (0·56 mm thick) was placed under each cage to collect urine. Pl. 1 shows the cages and funnels set up for collection.

Table 1. *Initial age of the lambs, live weight at the beginning of each period and the duration of the experimental periods*

	Lamb no.					
	101	106	107	108	109	111
Age at commencement of experiment (days)	35	16	8	21	20	5
Preliminary period (days)	10	14	14	9	9	13
Live weight (kg)	5·01	4·63	4·71	4·17	4·91	4·94
Nitrogen-free period (days)	8	14	12	12	10	12
Live weight (kg)	5·78	5·26	5·26	4·17	4·97	5·49
Recovery period (days)	5	7	10	8	8	9
Live weight (kg)	5·72	5·16	5·19	4·07	4·89	5·11

Faeces were collected in a plastic bag connected to a rubber collecting tube or funnel which was attached to the lamb by means of light canvas tapes. The construction of the harness is illustrated in Pl. 2. To make the rubber section, a motor scooter inner tube (size 3·50–8; about 20 cm inner diam.) was cut into eight sections about 11·5 cm long. Approximately 24 cm lengths of wire (diam. 3 mm) were made into ellipses of slightly larger diameter than could be obtained with the unstretched rubber tube (Pl. 2*A*). One of these wire rings was slipped over one end of an everted section of tubing which was then stretched out over the ring and sewn down, enclosing the ring and forming a flange. The tubing was everted so that its smooth surface would be the inside surface of the funnel. At the other end of the section of rubber tubing the short circumference side was cut back about 1·5 cm to make a closer fit with the hind-quarters of the lamb. A hole was cut in the long circumference side of the tube, about 2·5 cm from one end, to accommodate the tail of the lamb (Pl. 2*B* and *C*). The hole consisted of two cuts at right angles to each other and at 45° to the plane of the tube. Small holes punched at the ends of the cuts prevented tearing. This rubber funnel was held close to the lamb by tapes which were attached by tying through punched holes, three on the dorsal side near the aperture for the tail and two on the ventral side of the funnel (Pl. 2*C*). The two lower tapes were passed forward between the hind legs, crossed over the back and tied under the neck. The outer two of the upper three tapes were then passed along the back and around the crossed lower tapes, in such a way that they could not slip down, and then tied at the side after passing around the rib cage of the lamb. Finally, the central upper tape was drawn forward towards the head, to lift the funnel on the tail, and tied around the junction of the other tapes. This dorsal tape was found to be necessary to prevent the funnel slipping down under the hind-quarters of the lamb. The faeces were then collected in a weighed plastic bag (16·5 cm × 21 cm) held over the flange by an elastic rubber band. The complete harness is shown attached to a lamb in Pl. 3.

The disadvantages in the use of the harness were that the lambs began chewing the tapes from about 5 weeks of age onwards and, with liquid faeces, unless the bags were changed frequently, some degree of spilling occurred when the lambs lay down. The urine as voided was covered with a layer of toluene and the daily excretions of urine and faeces were stored at +5°. Bulk samples of urine were stored in Polythene bottles at −15° for subsequent analysis. Urinary N was determined daily. Faeces were dried at 60° under reduced pressure and ground to pass through a 1 mm sieve. The lambs were weighed to the nearest 10 g each morning before feeding.

Table 2. *Composition of the experimental diets expressed per* 100 *g dry matter*

Constituent	Diet no. 3	Diet no. 6*	N-free diet*
Spray-dried whole cow's milk (g)	100·0	39·0	—
Glucose (g)	—	22·2	47·2
Butter oil (g)	—	35·5	41·0
Glyceryl monostearate (g)	—	—	5·1
Minerals (g)	—	3·3	6·7
Crude protein (g)†	28·5	10·7	—
Ether extractives (g)	27·6	46·2	46·2
Ash (g)	5·9	5·6	6·6
Nitrogen-free extractives (by difference) (g)	38·0	37·5	47·2
Energy (kcal)‡	556	628	600
Protein calories as percentage of total calories	28·7	9·7	—

* See p. 191 for B-vitamin supplement.
† Crude protein (N × 6·38).
‡ Energy values (kcal/g on a 100% dry-matter basis): casein 5·575; dried whole cow's milk 5·558, glucose 3·736, butter oil 9·235, glyceryl monostearate 8·771.

Diets

The composition of the experimental diets is shown in Table 2. All diets contained 15% total solids and were prepared twice weekly. The lipid constituents of diet no. 6 and of the N-free diet were homogenized in a Weir Junior Homogenizer at a pressure of 850 lb/in² and at a temperature of 65–70°. The quantity of glyceryl monostearate added to the N-free diet was the minimum that would maintain the butter oil in a stable emulsion after homogenizing. The weight of sugar in the diets was controlled so that the total intake of glucose plus lactose, expressed in terms of hexose equivalent, did not exceed 9 g/kg live weight daily for each lamb (Walker & Faichney, 1964*b*). In the preliminary period the energy intake of the lambs was controlled at about 110 kcal/kg live weight 24 h, this quantity being based upon the live weight at the beginning of the experiment. The lambs were subsequently given the medium-protein diet in the recovery period at the same total energy intake, unadjusted for any change in live weight during the course of the experiment. The energy intake in the N-free period was adjusted to the live weight at the beginning of this period to supply about 110 kcal/kg live weight 24 h. The mean values for the energy intakes (kcal/kg 24 h) in the different periods, with their standard errors, were:

Preliminary period	113·5 ± 3·6
N-free period	109·2 ± 4·1
Recovery period	107·8 ± 7·3

A mineral mixture was added to diet no. 6 and to the N-free diet to adjust the mineral content of these diets more nearly to that of ewe's milk (Perrin, 1958). The composition of the mineral mixture, which was based upon that given to calves by Blaxter & Wood (1951*a*), is shown in Table 3 together with the mineral composition of the N-free diet.

Table 3. *Composition of the mineral mixture and comparison of the mineral content (mg/100 ml) of the nitrogen-free diet with that of ewe's milk*

Component	Quantity (g)	Component	Quantity (g)
$CaHPO_4$	137·90	$MnSO_4.4H_2O$	0·25
K_2HPO_4	137·50	$ZnCl_2$	0·25
$CaCl_2$	50·00	$CuSO_4.5H_2O$	0·25
MgO	20·00	$CoCl_2.6H_2O$	0·09
$Na_2HPO_4.12H_2O$	25·00	KI	0·50
NaCl	40·00	Ferric citrate	10·00
$CaCO_3$	50·00	NaF	0·25
$C_6H_8O_7.H_2O$	82·05	Water (final volume)	2 l.

	Na	K	Ca	Mg	P	Cl	Citrate
Ewe's milk*	41	132	186	18	175	129	—
N-free diet	41	134	178	26	132	123	175

* Perrin (1958).

A vitamin supplement containing certain members of the vitamin B complex was added to diet no. 6 and to the N-free diet so as to make their contents of these vitamins similar to those in ewe's milk; those vitamins whose concentrations in ewe's milk were not known were added in amounts similar to those in cow's milk. The B vitamin contents of the N-free diet and of ewe's milk are shown in Table 4. All lambs were dosed with 100000 i.u. vitamin A and 10000 i.u. ergocalciferol (1 ml) during the 2nd week of life and again 1 month later. Aureomycin soluble (0·45 g; Cyanamid of Great Britain, Ltd), which contained 25 mg chlortetracycline hydrochloride, was given daily dissolved in the milk to each lamb. A solution which contained $FeSO_4.7H_2O$, $CuSO_4.5H_2O$ and $CoCl_2.6H_2O$ was added to diet no. 3 so as to increase the concentration of the dry matter by 50 p.p.m. Fe, 50 p.p.m. Cu and 0·1 p.p.m. Co. The lambs were bottle-fed at 07.00, 14.00 and 22.00 h.

Analytical methods

Diet constituents. Determination of nutrients and of gross energy was the same as for faeces (see below); lactose was determined by the method of Shaffer & Hartmann (1920–1).

Urine. Total N was determined by the Kjeldahl method with copper and selenium as catalysts.

Table 4. *The B vitamin content of the nitrogen-free diet compared to that of ewe's milk (mg/l)*

Vitamin	N-free diet	Ewe's milk Value*	Ewe's milk Reference
Thiamine	0·6	0·63	1, 2
Riboflavine	5·0	4·99	1, 2, 3, 4
Nicotinic acid	5·1	5·13	1, 2, 3, 4
Choline	150·0	(147·0)	5
Pyridoxine	0·7	(0·7)	2
p-Aminobenzoic acid	0·1	(0·1)	6
Pantothenic acid	3·8	3·78	1, 2, 4

* Values in parentheses are for cow's milk.
(1) McGillivray (1949).
(2) Kon & Porter (1947–8).
(3) Moinuddin, Pope, Phillips & Bohstedt (1953).
(4) Ling, Kon & Porter (1961, Table 2).
(5) Griffith & Nyc (1954, Table 7).
(6) Wright & Tavormina (1954, Table 5).

Faeces. Total N was determined by the Kjeldahl method on the moist faeces, gross energy with the bomb calorimeter (Baird & Tatlock, London), dry-matter content by drying to constant weight at 60° under reduced pressure, ash by incineration at 600° and ether extractives by the method of Hopkins, Murray & Campbell (1955) modified as follows. The sample was extracted in a Soxhlet apparatus with light petroleum (b.p. 40–60°) for 8 h. Glacial acetic acid was added to the light petroleum in the ratio of 5:100 (v/v) and the mixture was heated to ensure complete mixing. After it had stood for 2 h, extraction was continued for a further 4 h. The solvent was removed by distillation and the extract was then dried to constant weight at 60° under reduced pressure.

Measurement of basal heat production

The closed-circuit respiration chamber of Alexander (1961) was used for the estimation of heat production by indirect calorimetry. Three estimations, each covering 2 h, were made on all lambs with an interval of about 25 min between them. The lambs were starved for 36 h before the estimations and were held in a lying position in the respiration chamber.

Endogenous urinary N

Lambs given the low-protein diet (no. 6) in the preliminary period excreted daily approximately one-half as much N in the urine as lambs given the medium-protein diet (no. 3). When the lambs were given the N-free diet the excretion of N fell rapidly for the first 3 days and more slowly thereafter, reaching a fairly constant level after 7 days. The daily collections of urine were stored separately but were bulked for 3 days once the N excretion had reached a low, yet constant, level. The lambs drank the N-free diet reasonably well, though most lambs would only finish the milk at a particular meal if they were taken out of their metabolism cages and handled, when they would suck eagerly. It was noticeable when the diet was changed from the N-free diet back to diet no. 3, in the recovery period, that the lambs quickly reverted to their previous habit of drinking readily from the bottle whilst in the cage.

Table 5. *Daily urinary nitrogen excretion* (g/24 h) of the lambs*

Day	Lamb no. 101	106	107	108†	109†	111
			Preliminary period			
1	1·68	1·77	2·48	1·02	1·16	2·29
2	1·80	1·83	2·40	1·02	1·07	2·27
3	**1·78**	**1·78**	**2·54**	**0·89**	**0·94**	**2·21**
4	**1·90**	**1·85**	**2·57**	**0·87**	**1·07**	**2·49**
5	**1·85**	**1·87**	**2·65**	**1·04**	**1·36**	**2·54**
6	—	2·00	2·74	—	—	2·49
7	—	1·89	2·77	—	—	2·79
			N-free period			
1	1·72	1·65	2·19	0·86	1·01	2·47
2	1·10	1·05	1·34	0·69	0·78	1·22
3	0·85	0·78	0·89	0·58	0·73	1·01
4	0·97	0·69	0·84	0·52	0·61	0·94
5	0·67	0·60	0·75	0·48	0·57	0·89
6	**0·72**	0·51	0·73	0·45	**0·48**	0·86
7	**0·64**	0·49	0·68	0·35	**0·54**	0·88
8	**0·75**	0·44	0·62	0·43	**0·62**	0·76
9	—	0·40	**0·67**	**0·41**	0·56	**0·87**
10	—	0·49	**0·58**	**0·42**	0·63	**0·85**
11	—	**0·42**	**0·54**	**0·46**	—	**0·76**
12	—	**0·42**	0·65	0·45	—	0·74
13	—	**0·44**	—	—	—	—
14	—	0·46	—	—	—	—
			Recovery period			
1	1·15	0·52	0·93	0·47	0·82	1·00
2	**1·78**	1·00	1·56	1·09	1·59	1·50
3	**1·94**	**1·69**	1·64	1·52	1·48	1·90
4	**1·86**	**1·65**	1·81	1·45	1·47	1·83
5	**1·97**	**1·66**	2·29	1·40	**1·48**	1·95
6	(2·42)	1·90	2·12	**1·43**	**1·60**	1·93
7	—	1·76	2·25	**1·38**	**1·67**	**1·76**
8	—	(2·16)	**2·29**	**1·25**	1·77	**1·88**
9	—	—	**2·38**	(1·93)	(2·07)	**1·94**
10	—	—	**2·42**	—	—	(2·29)
11	—	—	(2·48)	—	—	—

* Values in parentheses are for the N excretion during 24 h starvation; values in bold-face type are those taken for N balance; values boxed show the samples taken for N and S partition (Walker & Faichney, 1964*a*). The sequence of observations on each lamb was for consecutive days and the dashes in the vertical columns do not indicate missing values.

† Lambs nos. 108 and 109 were given the low-protein diet (no. 6) in the preliminary period.

Bulk faecal collections were made to correspond in time with the bulk urine collections, once the daily N excretion was at its lowest level. Lamb no. 106 remained eager for the N-free diet throughout the 14 days of the period, and was only taken off the diet to avoid unnecessary loss of weight.

It did not appear in this experiment that the two different levels of N intake in the preliminary period had any effect on the length of time taken to reach the lowest level of urinary N excretion on the N-free diet.

Table 6. *Nitrogen balance (g N/24 h) and live-weight change (g/24 h) of individual lambs in each period*

Lamb no.	N intake	Faecal N	Urinary N	N balance	Live-weight change
		Preliminary period			
101	4·31	0·13	1·80	+2·38	+77
106	4·04	0·34	1·86	+1·84	+45
107	4·35	0·03	2·59	+1·73	+39
108*	1·25	0·17	0·97	+0·11	0
109*	1·49	0·17	1·12	+0·20	+7
111	4·51	0·25	2·44	+1·82	+42
Mean*	4·30	0·19	2·17	+1·94	+51
		N-free period			
101	0·00	0·32	0·70	−1·02	−8
106	0·00	0·35	0·48	−0·83	−7
107	0·00	0·13	0·59	−0·72	−6
108	0·00	0·17	0·43	−0·60	−8
109	0·00	0·34	0·55	−0·89	−8
111	0·00	0·36	0·83	−1·19	−32
Mean	0·00	0·27	0·57	−0·84	−11
		Recovery period			
101	4·31	0·29	1·94	+2·08	+79
106	4·04	0·41	1·71	+1·92	+65
107	4·35	0·10	2·43	+1·82	+69
108	3·58	0·26	1·35	+1·97	+72
109	4·30	0·25	1·51	+2·54	+110
111	4·55	0·51	1·90	+2·14	+97
Mean	4·12	0·29	1·71	+2·12	+82

* Values for lambs nos. 108 and 109 fed on diet no. 6 were omitted from the mean values of the preliminary period.

The urinary excretion of N on a N-free diet can be considered to be solely of endogenous origin only if the energy intake of the animal is adequate. If the energy intake is insufficient, body protein will be katabolized to provide energy and the content of N in the urine will be increased above the endogenous level by N resulting from the breakdown of body protein. This increase in urinary N was readily seen when the lambs were starved before the measurement of the basal heat production. All lambs showed a similar negative N balance during the N-free period, which suggests that the energy intake was adequate. Since all the food offered during this period was consumed, the values for urinary N excretion may be considered to be truly endogenous. The daily urinary N excretions of all lambs are given in Table 5.

N balance

Details of the individual N balances are given in Table 6. Values were based on bulk samples taken over a 5- or 7-day period in the preliminary period and usually over the last 3 days of the recovery period. In both the preliminary and recovery periods all lambs were in positive N balance. The values given in Table 6 for the N-free period are based on a 3-day sample of urine and faeces.

Live-weight gain or loss

Daily weight changes were irregular and a carry-over effect was noticed when dietary changes were made. The live-weight changes are shown in Table 6. These values were based on regression analyses of the daily weights, omitting values obtained on the 2 days after a change in diet. There was no significant difference between the mean live-weight gains of lambs given diet no. 3 in the preliminary and recovery periods. Lambs given the low-protein diet (no. 6) failed to gain weight, although their intake of energy was similar to that of lambs given the medium-protein diet (no. 3).

Table 7. *Mean digestibility (%) for the different nutrients with their standard errors*

No. of lambs	Diet no.	Dry matter	Energy	Crude protein	Ether extractives	Nitrogen-free extractives
			Preliminary period			
4	3	97·1 ± 1·0	97·8 ± 0·9	95·7 ± 1·7	98·5 ± 0·6	98·3 ± 0·8
2	6	95·2 ± 1·9	95·5 ± 1·9	87·9 ± 0·9	96·4 ± 1·6	96·9 ± 2·2
			N-free period			
6	N-free	92·7 ± 0·7	95·0 ± 1·0	—	94·7 ± 0·6	97·9 ± 0·3
			Recovery period			
6	3	94·8 ± 0·6	96·7 ± 0·5	92·9 ± 1·3	98·1 ± 0·4	97·3 ± 0·4

Digestibility coefficients

The mean coefficients of digestibility of dry matter, of energy and of individual nutrients are given in Table 7, together with their standard errors. The digestibility of dry matter and ether extractives was significantly reduced when the lambs were given the N-free diet. No other differences were significant.

The decreased digestibility of the dry matter and ether extractives when the lambs were given the N-free diet was accompanied by an increase in the excretion of water in the faeces though, owing to the range of values, the mean differences were not significant. When expressed as g water/1000 ml liquid ingested, the mean values with their ranges were:

Preliminary period	12·8 (1·9–33·3)
N-free period	35·5 (10·6–60·2)
Recovery period	19·7 (6·1–39·9)

Although the intake of fat by the two lambs given the low-protein diet (no. 6) in the preliminary period was almost the same as that during the N-free period, there was an increase in water excreted in the latter period, which suggests that the high fat intake alone was not responsible for the increased excretion of water and lower digestibility.

The intake of hexose equivalent (glucose plus lactose, expressed as hexose sugar) with the N-free diet was significantly greater than with diet no. 3, though the intake with all diets was below that which is believed to be responsible for diarrhoea in lambs receiving experimental diets (Walker & Faichney, 1964*c*). The mean values and their ranges, expressed as g hexose equivalent/kg live weight 24 h were:

Preliminary period	7·2 (6·9–7·5)
N-free period	8·8 (8·2–9·4)
Recovery period	7·1 (6·6–7·9)

Table 8. *Mean basal heat production of individual lambs*

Lamb no.	Live weight (kg)	Surface area* (m^2)	Temperature: Rectal (°C)	Temperature: Chamber (°C)	R.Q.	Oxygen consumed (l./h)	Heat production: kcal/h	Heat production: kcal/kg h	Heat production: kcal/m^2 h
101	5·63	0·336	40·0	30·6	0·73	3·80	17·9	3·2	53·3
106	5·41	0·328	40·3	30·0	0·72	3·66	17·3	3·2	52·7
107	5·72	0·339	39·4	29·6	0·81	3·06	14·7	2·6	43·4
108	4·36	0·289	39·5	31·0	0·75	2·45	11·7	2·7	40·4
109	5·24	0·322	39·7	30·4	0·74	3·00	14·2	2·7	44·1
111	5·49	0·331	39·4	31·3	0·74	3·05	14·4	2·6	43·6

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

Basal heat production

Table 8 gives the basal heat production of the lambs. Since the respiration chamber was designed originally for newborn lambs it was not possible to accommodate lambs weighing over about 6·5 kg. The lambs rested quietly in the chamber throughout the period of the measurements.

DISCUSSION

Metabolic faecal N

The N excreted in the faeces when an animal is given a N-free diet represents an unavoidable loss of N to the body, though the magnitude of the loss is affected by the amount of dry matter eaten (Mitchell, 1926; Schneider, 1934) and by the size of the animal (Lofgreen & Kleiber, 1953). The mean metabolic faecal N values obtained in our experiment were:

Metabolic faecal N (g/100 g dry-matter intake)	0·29 (range 0·13–0·37)
Metabolic faecal N (g/100 g dry matter excreted)	3·86 (range 2·61–4·46)

In Table 9 are given values obtained by other workers for the metabolic faecal N excretion of the adult sheep. A mean value of 0·54 g N/100 g dry-matter intake may be compared with the mean value of 0·29 g for lambs in our experiment. No previous experiments have been reported in which the metabolic faecal N excretion of young lambs in the preruminant stage of development has been determined. Blaxter & Wood (1951*a*) found that the metabolic faecal N excretion of three milk-fed calves was

0·43 g/100 g dry matter ingested, and in a similar experiment with four calves Cunningham & Brisson (1957) reported a value of 0·33 g/100 g dry matter consumed.

These values for the young ruminant are higher than the average value for the adult non-ruminant, which is generally taken to be approximately 0·1 g N/100 g dry matter consumed (Maynard & Loosli, 1962). The reason for this difference is not readily apparent.

Table 9. *Metabolic faecal nitrogen (g/100 g dry-matter intake) excretion of adult sheep*

Value	Reference
0·58–0·75	Sotola (1930)
0·56	Turk, Morrison & Maynard (1934)
0·55	Harris & Mitchell (1941)
0·51	Morgen, Beger & Westhauser (quoted by Mitchell, 1926)
0·50	Smuts, Marais & Bonsma (1940)
0·45	Hutchinson & Morris (1936)

The weight of faecal N excreted daily/100 g dry matter ingested by lambs given diet no. 3, both in the preliminary and in the recovery periods (0·26 g) was on average less than that of faecal N excreted in the N-free period (0·29 g). In a subsequent experiment (Walker & Faichney, 1964*c*) when diet no. 3 was given to twenty-four lambs at a level of energy intake similar to that in the experiment described here, the mean daily excretion of faecal N was 0·23 g/100 g dry matter consumed. However, these differences were not significant and merely served to confirm the high digestibility of the protein in dried whole cow's milk, without necessarily suggesting that values for metabolic faecal N, determined on a N-free diet, are unreliable when compared with values obtained when a small amount of a highly digestible protein is eaten (cf. Mitchell & Carman, 1926; Bosshardt & Barnes, 1946).

N balance and live-weight gain

The values for N balance were related to live-weight gain, when both were expressed as g/kg live weight weekly. The values for the ten lambs given diet no. 3 were considered separately from those for lambs given the other two diets. The regression equation for the lambs given diet no. 3, with the residual standard deviation, was

$$NB = 0{\cdot}0118G + 1{\cdot}61\ (\pm 0{\cdot}26), \tag{1}$$

where NB = N balance (g N)/kg live weight week and G = gain (g)/kg live weight week. In a subsequent experiment (Walker & Faichney, 1964*c*) when twenty-four lambs were given diet no. 3, the regression equation relating these two variables, with the residual standard deviation, was:

$$NB = 0{\cdot}0147G + 1{\cdot}80\ (\pm 0{\cdot}45). \tag{2}$$

The slopes of the two regression lines were in fact very similar and did not differ significantly; the points at which the lines intercepted the Y axis were also not significantly different. The mean gain in weight with the standard error, for lambs given diet no. 3 in the experiment described here, was 93·1 ± 10·5 g/kg week compared with 114·6 ± 7·1 in the subsequent experiment. The difference was not significant.

Endogenous N and basal heat production

It has already been established that the endogenous urinary N values obtained in this experiment were almost certainly representative of a state in which the energy intake was adequate for the maintenance of body tissue. The requirements for the measurement of the basal heat production were probably also satisfied by the conditions of the experiment (cf. Blaxter & Wood, 1951*b*; Roy, Huffman & Reineke, 1957). The values obtained for urinary N excretion with zero N intake and for basal heat production are given in Tables 6 and 8 respectively. The mean value with the standard error for the six lambs was 1·67 ± 0·17 mg N/kcal basal heat production. There are no published values for young lambs, though the value of 1·90 mg N/kcal reported for milk-fed calves by Blaxter & Wood (1951*a*) is not dissimilar. Smuts (1935) found that the ratio of endogenous urinary N to the basal heat production of mature animals of several different species was 2·00 mg N/kcal.

Table 10. *Relation between published values for the endogenous urinary nitrogen excretion of sheep and the calculated basal heat production*

Weight (kg)	Endogenous N (mg/kg 24 h)	Basal heat production* (kcal/kg 24 h)	Relation (mg N/kcal)	Reference
35·0	68	32·4	2·12	Morgen *et al.* (1911)†
45·0	58	28·1	2·07	
45·0	54	28·1	1·93	
35·0	52	32·2	1·62	
40·0	48	30·1	1·60	
40·0	43	30·1	1·43	
47·0	72	27·3	2·64	Morgen *et al.* (1914)†
38·0	68	31·0	2·20	
43·5	55	28·6	1·92	
42·0	44	29·2	1·51	
54·0	38	24·9	1·53	
33·1	31	33·3	0·93	Scheunert, Klein & Steuber (1922)†
43·5	27	28·6	0·94	
44·1	24	28·4	0·85	
22·3	52	39·4	1·32	Smuts & Marais (1939)
41·9	44	29·3	1·50	
24·3	33	38·1	0·87	Sotola (1930)
23·5	52	38·6	1·35	Turk *et al.* (1934)
27·5	44	36·3	1·21	
36·5	35	31·6	1·11	
31·9	31	33·9	0·92	Völtz (1920)†
Mean	46	31·4	1·52	

* Calculated from the equation of Brody (1945, p. 468) for sheep (wethers):

$$\frac{Qb}{m} = 47e^{-0.022m} + 10{\cdot}6,$$

where Qb = basal heat production (kcal/24 h) and m = live weight (kg).

† Quoted by Mitchell (1929).

No experiments with adult sheep have been reported in which both the endogenous N excretion and the basal heat production have been determined on the same animal, though there are many reports of their separate determination in different sheep. In Tables 10 and 11 some of the published values are given and the undetermined variable has been calculated. The mean values were 1·52 and 1·87 mg N/kcal respectively, neither of which differed significantly from the value of 1·67 determined with milk-fed lambs in our experiment. However, Blaxter (1962*a*) has suggested that in the earlier determinations of basal heat production by Ritzman & Benedict (1930, 1931) and by Lines & Peirce (1931) the period of fasting was too short and therefore the heat production was above the basal level. By applying corrections to the earlier

Table 11. *Relation between published values for the basal heat production of sheep and the calculated endogenous urinary nitrogen excretion*

Weight (kg)	Basal heat production (kcal/kg 24 h)	Endogenous N* (mg/kg 24 h)	Relation (mg N/kcal)	Reference
28·7	23	57	2·48	Blaxter (1948)
40·3	23	52	2·26	Blaxter & Graham (1955)
60·0	19	46	2·44	Brody (1945)
70·0	21	44	2·12	
24·6	38	59	1·57	Lines & Peirce (1931)
35·6	29	54	1·85	
40·0	34	52	1·53	
40·4	25	52	2·07	Marston (1948)
6·6	108†	86†	0·80†	Peirce (1934)
27·8	41	58	1·40	
49·6	27	49	1·81	
52·9	33	48	1·46	
46·6	27	50	1·84	Ritzman & Benedict (1931)
47·4	25	50	1·98	Ritzman & Benedict (1930)
Mean	28	52	1·87	

* Calculated from the equation of Swanson & Herman (1943):

$$EUN = 0{\cdot}146X^{0{\cdot}72},$$

where EUN = endogenous urinary N (g/24 h) and X = live weight (kg).

† Omitted from the mean.

values so that a standard period of fasting of 64 h applied to all results, a basal heat production of 20·6 kcal/kg live weight 24 h was obtained (Blaxter, 1962*a*). This value is much lower than the mean value of 31·4 kcal/kg 24 h shown in Table 10 or of 28·0 kcal in Table 11. If this corrected mean for the basal heat production is related to the determined endogenous N value in Table 10 or to the calculated value in Table 11, then 2·23 and 2·52 respectively are obtained, values much higher than the mean of 1·67 for the milk-fed lambs. Blaxter (1962*b*) has also recalculated some of the results of Ritzman & Benedict (1930, 1931) for the basal heat production of the young lamb at various ages from 1 week up to 9 weeks. Blaxter stated that the fasting heat production was overestimated by 35%. When these values, which were computed to a 'true fasting value', were expressed/kg live weight 24 h the basal heat production at different ages was: 82 kcal (1 week), 61 kcal (3 weeks), 59 kcal (6 weeks) and 53 kcal

(9 weeks). These values compare with a mean of 68 kcal for lambs aged between 5 and 8 weeks in our experiment, and with a value of 60 kcal for unsuckled single lambs during starvation (Alexander, 1962).

SUMMARY

1. Six male Merino lambs were each given a nitrogen-free liquid diet for periods varying from 8 to 14 days during the first 8 weeks of life. Quantitative collections of urine and faeces were made daily.

2. A medium-protein (28·5% on a dry-matter basis) or low-protein (10·7%) diet was given in a preliminary period and the medium-protein diet in a recovery period. Measurements of basal heat production were made after the animals had been starved for 36 h at the end of the recovery period.

3. Metabolic faecal N excretion varied from 0·13 to 0·37 g/100 g dry matter consumed, with a mean value of 0·29 g.

4. The mean value for the endogenous urinary N excretion was 111·8 mg/kg live weight 24 h and for the basal heat production 67·8 kcal/kg live weight 24 h; the mean endogenous N per kcal basal heat production was 1·67 mg.

5. Digestibility of the dry matter was lowest with the N-free diet owing mainly to a lower digestibility of the ether extractives. The digestibility of energy and of the N-free extractives was unchanged.

6. Live-weight maintenance was associated with a N balance of +1·61 g/kg live weight week.

We wish to thank Mr D. Williams of the Division of Animal Physiology, CSIRO, Prospect, NSW, Australia, for the measurements of basal heat production.

REFERENCES

Alexander, G. (1961). *Aust. J. agric. Res.* **12**, 1139.
Alexander, G. (1962). *Aust. J. agric. Res.* **13**, 144.
Ashworth, U. S. (1935). *Res. Bull. Mo. agric. Exp. Sta.* no. 223.
Ashworth, U. S. & Cowgill, G. R. (1938). *J. Nutr.* **15**, 73.
Blaxter, K. L. (1948). *J. agric. Sci.* **38**, 207.
Blaxter, K. L. (1962*a*). *Brit. J. Nutr.* **16**, 615.
Blaxter, K. L. (1962*b*). *The Energy Metabolism of Ruminants*, p. 96. London: Hutchinson.
Blaxter, K. L. & Graham, N. McC. (1955). *J. agric. Sci.* **46**, 292.
Blaxter, K. L. & Mitchell, H. H. (1948). *J. Anim. Sci.* **7**, 351.
Blaxter, K. L. & Wood, W. A. (1951*a*). *Brit. J. Nutr.* **5**, 11.
Blaxter, K. L. & Wood, W. A. (1951*b*). *Brit. J. Nutr.* **5**, 29.
Bosshardt, D. K. & Barnes, R. H. (1946). *J. Nutr.* **31**, 13.
Brody, S. (1945). *Bioenergetics and Growth.* New York: Reinhold Publishing Corp.
Cunningham, H. M. & Brisson, G. J. (1957). *Canad. J. Anim. Sci.* **37**, 152.
Griffith, W. H. & Nyc, J. F. (1954). In *The Vitamins.* Vol. 2, p. 59. [W. H. Sebrell Jr. and R. S. Harris, editors.] New York: Academic Press Inc.
Harris, L. E. & Mitchell, H. H. (1941). *J. Nutr.* **22**, 167.
Hopkins, C. Y., Murray, T. K. & Campbell, J. A. (1955). *Canad. J. Biochem.* **33**, 1047.
Hutchinson, J. C. D. & Morris, S. (1936). *Biochem. J.* **30**, 1682.
Kon, S. K. & Porter, J. W. G. (1947–8). *Nutr. Abstr. Rev.* **17**, 31.
Lines, E. W. & Peirce, A. W. (1931). *Bull. Coun. sci. industr. Res. Aust.* no. 55.
Ling, E. R., Kon, S. K. & Porter, J. W. G. (1961). In *Milk: The Mammary Gland and its Secretion.* Vol. 2, p. 195. [S. K. Kon and A. T. Cowie, editors.] New York: Academic Press Inc.
Lofgreen, G. P. & Kleiber, M. (1953). *J. Nutr.* **49**, 183.

McGillivray, W. A. (1949). *J. agric. Sci.* **39**, 143.
Marston, H. R. (1948). *Aust. J. sci. Res.* B, **1**, 93.
Maynard, L. A. & Loosli, J. K. (1962). *Animal Nutrition*, 5th ed., p. 111. Toronto: McGraw-Hill Book Company Inc.
Mitchell, H. H. (1926). *Bull. nat. Res. Coun., Wash.*, no. 55.
Mitchell, H. H. (1929). *Bull. nat. Res. Coun., Wash.*, no. 67.
Mitchell, H. H. & Carman, G. G. (1926). *J. biol. Chem.* **68**, 183.
Moinuddin, M., Pope, A. L., Phillips, P. H. & Bohstedt, G. (1953). *J. Anim. Sci.* **12**, 497.
Morgen, A., Beger, C. & Westhauser, F. (1911). *Landw. VersSta.* **75**, 265.
Morgen, A., Beger, C. & Westhauser, F. (1914). *Landw. VersSta.* **85**, 1.
Peirce, A. W. (1934). *Bull. Coun. sci. industr. Res. Aust.* no. 84.
Perrin, D. R. (1958). *J. Dairy Res.* **25**, 70.
Ritzman, E. G. & Benedict, F. G. (1930). *Tech. Bull. N.H. agric. Exp. Sta.* no. 43.
Ritzman, E. G. & Benedict, F. G. (1931). *Tech. Bull. N.H. agric. Exp. Sta.* no. 45.
Roy, J. H. B., Huffman, C. F. & Reineke, E. P. (1957). *Brit. J. Nutr.* **11**, 373.
Scheunert, A., Klein, W. & Steuber, M. (1922). *Biochem. Z.* **133**, 137.
Schneider, B. H. (1934). *Biochem. J.* **28**, 360.
Shaffer, P. A. & Hartmann, A. F. (1920–1). *J. biol. Chem.* **45**, 365.
Smuts, D. B. (1935). *J. Nutr.* **9**, 403.
Smuts, D. B. & Marais, J. S. C. (1939). *Onderstepoort J. vet. Sci.* **13**, 219.
Smuts, D. B., Marais, J. S. C. & Bonsma, J. C. (1940). *Onderstepoort J. vet. Sci.* **15**, 211.
Sorg-Matter, H. (1928). *Arch. int. Physiol.* **30**, 126.
Sotola, J. (1930). *J. agric. Res.* **40**, 79.
Swanson, E. & Herman, H. A. (1943). *Res. Bull. Mo. agric. Exp. Sta.* no. 382.
Treichler, R. (1939). The relationship between basal metabolism and endogenous nitrogen metabolism. Ph.D. Thesis, University of Illinois.
Turk, K., Morrison, F. B. & Maynard, L. A. (1934). *J. agric. Res.* **48**, 555.
Völtz, W. (1920). *Biochem. Z.* **102**, 151.
Walker, D. M. & Faichney, G. J. (1964*a*). *Brit. J. Nutr.* **18**, 201.
Walker, D. M. & Faichney, G. J. (1964*b*). *Brit. J. Nutr.* **18**, 209.
Walker, D. M. & Faichney, G. J. (1964*c*). *Brit. J. Nutr.* **18**. (In the Press.)
Wright, L. D. & Tavormina, P. A. (1954). In *The Vitamins*. Vol. 3, p. 27. [W. H. Sebrell Jr. and R. S. Harris, editors.] New York: Academic Press Inc.

EXPLANATION OF PLATES

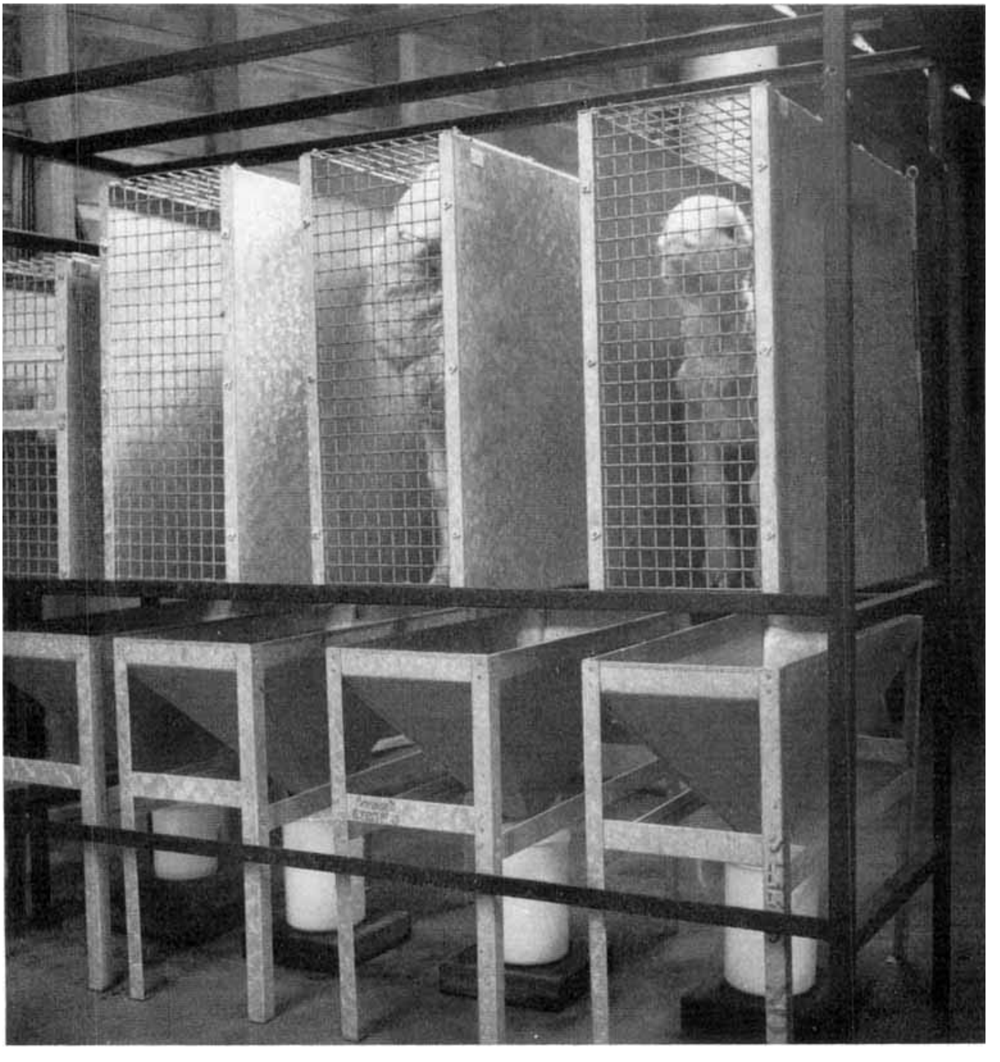
Pl. 1. Lamb metabolism cages with metal funnels in position for urine collection.

Pl. 2. Component parts of harness for collection of faeces of lambs.

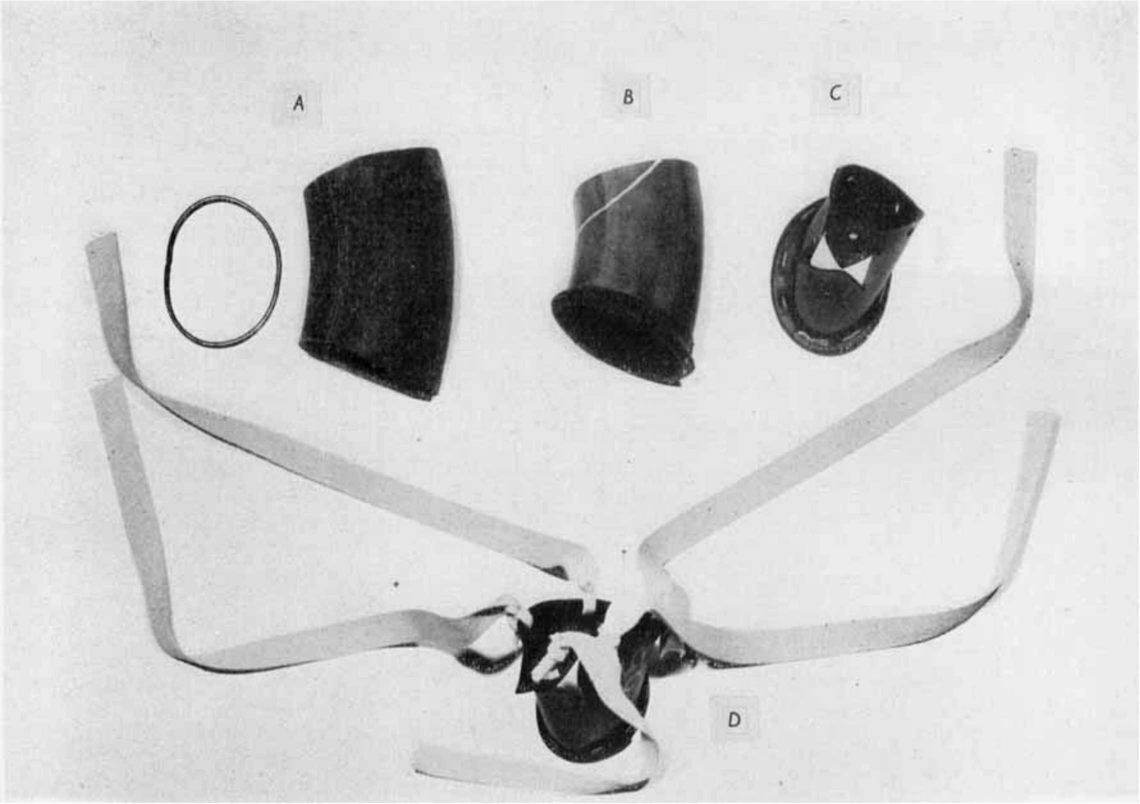
A. Metal ring and section of motor-scooter tube.
B. Metal ring sewn into everted rubber tube; white mark shows area to be removed to allow harness to fit snugly around hind-quarters.
C. Tail hole and eyelets for harness tapes.
D. Completed harness.

Pl. 3. Lamb fitted with harness for collection of faeces.

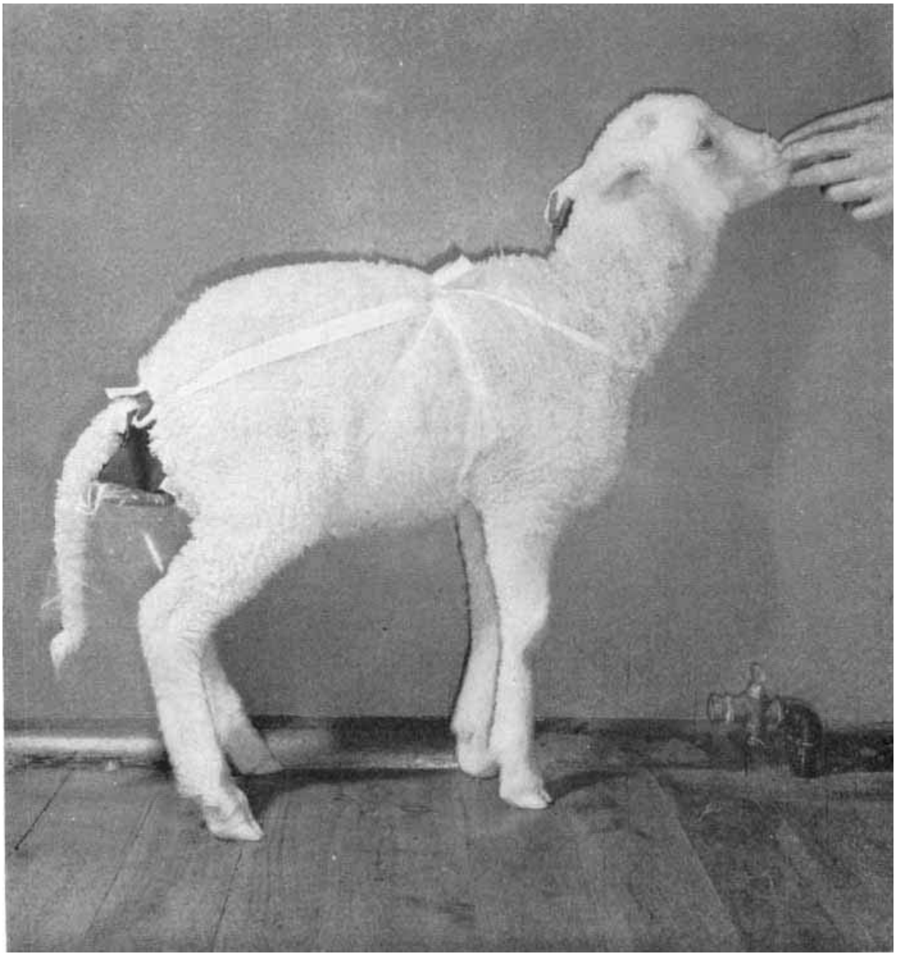
Printed in Great Britain



D. M. WALKER AND G. J. FAICHNEY (*Facing p.* 200)



D. M. WALKER AND G. J. FAICHNEY



D. M. WALKER AND G. J. FAICHNEY