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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Eighty-third Meeting of the Nutrition Society (One Hundred and Fiftieth of the Scottish Group) was held in the Craigie College of Education, Ayr, on Friday, 8 *April* 1983, *when the following papers were read:*

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Zinc supplementation in urban Amazonian mothers: concentrations of Zn and retinol in maternal serum and milk. By R. SHRIMPTON, H. A. MARINHO, Y. S. ROCHA and F. H. ALENCAR, *Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil*

Zinc intakes and serum Zn levels are known to be low in Manaus (Shrimpton, 1980). Although great geographic variations are observed in human milk Zn concentrations, human studies have not corroborated animal evidence that milk Zn levels are dependent on maternal Zn status. A Zn supplementation study was carried out in Manaus on poor mothers, solely breast-feeding their infants until 5 months of age. Since Zn status influences vitamin A status, both nutrients were investigated. A double-blind study, initially involving seventy-nine mothers, was performed by distributing capsules containing 15 mg Zn, or a placebo, for daily consumption. At 30 and 120 d post partum, a portion of milk was collected by manual expression and blood obtained by intravenous puncture, taking precautions to avoid Zn contamination and retinol destruction. Serum and milk Zn were determined using atomic absorption spectrophotometry and retinol by using a modified Bessey–Lowry method.

Zinc and vitamin A in milk and serum of poor Amazonian mothers

	30 d post partum						120 d post partum					
	Zn supplemented			Placebo			Zn supplemented			Placebo		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Serum Zn (μg/l)	33	965*	111	33	940	143	21	1017*†	88	24	960†	111
Serum retinol (μg/l)	33	410	164	31	383	166	25	473†	151	16	368†	156
Milk Zn (μg/l)	29	1870	1170	30	2080**	1280	29	1410	780	19	1330**	540
Milk retinol (μg/l)	30	466†††	176	25	350**†††	174	28	525†††	149	18	238**†††	78

Significant difference between time means: *$P<0.05$, **$P<0.01$.
Significant difference between treatment means: †$P<0.05$, †††$P<0.001$.

The results suggest that mothers took their capsules and that improvement in Zn status was associated with an improvement in vitamin A status. Milk retinol levels of Zn-supplemented mothers were similar to levels reported for UK mothers, whereas by 120 d these levels were almost half those observed in non-supplemented mothers. Milk Zn levels were not significantly different at 30 or 120 d; at 30 d, levels were half those reported for UK mothers, but similar to most other studies at 120 d. Milk Zn levels fell significantly from 30 to 120 d in non-supplemented mothers. These results suggest that maternal Zn status is an important factor determining vitamin A status of breast-fed Amazonian infants at weaning, and that other factors besides dietary Zn are responsible for the observed geographic variation in human milk Zn concentrations in early lactation.

This research was funded by the Brazilian Council for Scientific and Technological Development (CNPq).

Shrimpton, R. (1980). Studies on zinc nutrition in the Amazon Valley. PhD Thesis, University of London.

Zinc supplementation and secretory immunoglobulin production in non-breast-fed Amazonian infants. By K. LEHTI,* R. SHRIMPTON,† F. H. ALENCAR† and J. C. WATERLOW,* **London School of Hygiene and Tropical Medicine, Keppel Street, London WC1* and †*Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil*

Previous surveys in urban Amazonian populations have indicated that zinc intake in non-breast-fed infants was 46% of the recommended intake (Shrimpton, 1980). Since Zn deficiency exerts a profound effect over the immune system, and secretory immunoglobulin A (SIgA) production is important in protecting against diarrhoea (common in non-breast-fed infants) an investigation was carried out to determine the effect of Zn supplementation on SIgA production in such children in a poor peri-urban slum area of Manaus.

After weighing and measuring seventy non-breast-fed children between 3 and 15 months of age, obtained by a house-to-house search, twenty-two pairs of children were obtained who were the same sex, within 2 months of age, within 5% of height for age and above 80% weight for height. Zn was added double-blind to half of the multivitamin solutions, randomly distributed to these children, such that one of each pair received approximately 5 mg Zn/d during the 3 month study period. Saliva, urine and faeces were collected at the beginning, the end and twice during the study period. SIgA was determined in saliva by radial-immunodiffusion. Zn was determined in faeces and urine by atomic absorption spectrometry. Urinary creatinine was determined by the picrate method. The data were analysed using a paired *t* test.

Urinary Zn:creatinine values were significantly higher in boys receiving Zn ($P<0.025$) but not girls. Faecal Zn:ash values were significantly higher in Zn-supplemented girls ($P<0.05$) but not boys. Salivary SIgA levels were significantly higher in Zn-supplemented boys ($P<0.005$) but not girls. The low urinary Zn:creatinine values in non-Zn-supplemented boys would suggest that they were Zn deficient, since urinary Zn is only reduced in Zn deficiency. The significant increase in faecal Zn in Zn-supplemented girls, but not boys, would suggest that boys utilized the supplement more than the girls. Whether the positive effect of Zn supplementation on SIgA production in boys observed in this study is capable of influencing diarrhoea occurrence needs further investigation. The apparent sex difference observed in Zn requirements may be associated with the higher rates of mortality and morbidity observed in male infants.

This research was funded by the ODA and the Brazilian Council for Scientific and Technological Development (CNPq).

Shrimpton, R. (1980). Studies on zinc nutrition in the Amazon Valley. PhD Thesis, University of London.

Qualitative assessment of milk intake in suckling mice by a radio-isotopic method. By C. C. THORNBURN and C. J. BAILEY (Introduced by Dr. C. B. COWEY), *Department of Biological Sciences, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET*

A radioisotopic method to assess milk intake in suckling mice was investigated. A γ- or hard β-emitting isotope is injected into the mother, transferred to the litter during suckling, and determined in the pups by whole-body counting.

^{125}Iodine was a suitable isotope; 2·5 μCi in 0·1 ml saline (9 g sodium chloride/l), injected subcutaneously dorso-cervically, gave a count rate of 5000–10 000 counts/min in the pups. Radioactivity was detected in the pups at 1 h after injection; it increased rapidly for approximately 6 h, then levelled off, and after 20 h declined exponentially. This pattern was observed in pups between 6 h and 15-d-old, with good repeatability among pups within a litter and between different litters. Experiments where suckling was prevented demonstrated negligible transfer of activity from mother to litter except via the milk. An acrylic skin dressing (Nobecutane®, Astra Chemicals, Watford) was applied over the nipples immediately before injecting ^{125}I; radioactivity in mother and pups was counted at intervals and the pups individually weighed for a 12-h period. The sealant was removed with solvent at 4 h. Initially, radioactivity in the pups was <0·1% of that injected into the mother, and their weight decreased slightly; after the sealant was removed, allowing the pups to suckle, their radioactivity rose rapidly, correlating closely with the weight gained.

Recirculation of isotope from pups to mother was shown by injecting each pup with 0·1 μCi ^{125}I in 0·025 ml saline and counting the radioactivity in mother and pups. The pattern of transfer varied between litters, but usually reached a plateau at 6–8 h, when the mother's radioactivity averaged 17% of the total injected into the pups. The sum of the mother's radioactivity and that remaining in the litter accounted for 99% of the original injection, over the 8-h period studied. Evidence for recirculation was also seen when pups were transferred to a non-radioactive foster-mother 24 h after their natural mother had been injected with the isotope. The pups lost radioactivity exponentially, rapidly for 3–5 h (half-life 9·8 (SE 0·9) h) and subsequently more slowly (half-life 27 (SE 2) h).

The method described is simple and rapid, allowing frequent determinations with minimal stress to mother and pups. It could be extended to study the fate of a variety of substances in a suckling litter.

Fatty acid composition of milk lipids from lactating cows maintained by intragastric infusion. By. A. K. LOUGH, E. R. ØRSKOV, A. G. CALDER and LESLEY COUTTS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Intragastric infusion of volatile fatty acids and casein (Ørskov *et al.* 1979) to cows in lactation has enabled a study to be made of the effect of intake of these acids on the fatty acid composition of milk without the influence of dietary lipids and those of rumen micro-organisms.

Two cows were given the nutritional treatments shown in the Table. Fatty acid composition of total milk lipids was determined by gas–liquid chromatography of butyl esters (Gander *et al.* 1962) and methyl esters (Smith *et al.* 1979).

All the milk lipids contained the normal complement of fatty acids, C_4–C_{18}. For infusions containing 75 and 65% acetate respectively (see Table), the milk fatty acid compositions were normal (Gander *et al.* 1962).

% Fatty acid in infusate (molar %)			% Fatty acid in milk lipid				Milk lipid
C_2	C_3	*n*-C_4	C_{16}	C_{18}	Odd-chain	Branched-chain	(g/d)
75	15	10	45	32	2	1·1	547
65	25	10	41	31	3	1·0	504
55	35	10	51	19	4	0·8	396
45	45	10	45	16	10	0·5	189
			30*	46*	3*	3·3*	631*

*Values for a cow given hay and concentrates.

With increasing proportions of propionate in the infusate the content of odd-chain fatty acids increased to as much as one-tenth of the total fatty acids, presumably as a result of increased availability of the primer unit, propionyl CoA (James *et al.* 1956). No branched acids, such as could be attributed to the utilization for fatty acid synthesis of the propionate metabolite methylmalonate (see Smith *et al.* 1979), were detected in the milk samples.

In the Table 'branched-chain' refers solely to the total content of *iso* and *anteiso* acids. Since these are ultimately of ruminal bacterial origin it is not surprising that the relative proportion of these acids is vastly greater in the animal given hay and concentrates than in the animals maintained by intragastric infusion.

Gander, C. W., Jensen, R. G. & Sampugna, J. (1962). *J. Dairy Sci.* **45**, 323.
James, A. T., Peeters, G. & Lauryssens, M. (1956). *Biochem. J.* **64**, 726.
Ørskov, E. R., Grubb, D. A., Wenham, G. & Corrigall, W. (1979). *Br. J. Nutr.* **43**, 553.
Smith, A., Calder, A. G., Lough, A. K. & Duncan, W. R. H. (1979). *Lipids* **14**, 953.

The digestibilities of milk fat and milk protein in lambs receiving fresh herbage. By R. W. MAYES and P. M. COLGROVE, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY*

During the first 4 months of life most lambs receive a diet of both milk and herbage. Little is known of the effects of age, ingestion of herbage and level of milk intake upon the utilization of nutrients supplied by milk.

In order to establish if milk digestibility can be affected by ingestion of herbage and alteration in milk intake, eight male lambs, initially aged 2 weeks, were offered ewe milk replacer, containing skimmed cows' milk and butterfat (298 g crude protein/kg dry matter (DM), 266 g fat/kg DM), at two declining feeding levels, for four consecutive 21-d periods. After the first period the lambs were given free access to fresh ryegrass/clover herbage (in vitro organic matter (OM) digestibility, 0·75 (SE 0·007)) which was cut daily. Ewes' milk containing ^{3}H-labelled fat and protein was incorporated into the liquid feeds for the final 10 d and faeces were collected over the final 5 d of each period. The ^{3}H levels in extracts and residues from Soxhlet determinations (solvent: 90 ml glacial acetic acid/l in petroleum ether (b.p. 60–80°)) of feeds and faeces were used to obtain respective digestibility values for [^{3}H]milk fat and [^{3}H]milk protein. The apparent digestibilities of total dietary fat and nitrogen were also determined.

Whilst the level of milk intake within each period had no effect on digestibility or herbage intakes, changes with time are shown in the Table.

Period . . .	1	2	3	4	Significance
Age (weeks)	4	7	10	13	—
Milk intake (g OM/d)	306	264	205	143	—
Herbage intake (g OM/d)	—	9·6	27·1	87·9	••
Apparent digestibility of:					
[^{3}H]milk fat	0·994	0·993	0·989	0·976	•••
[^{3}H]milk protein	0·985	0·984	0·966	0·961	•••
Total dietary fat	0·989	0·980	0·957	0·887	•••
Total dietary N	0·968	0·958	0·901	0·855	•••

••$P<0·01$, •••$P<0·001$.

As expected, there were progressive reductions in total dietary fat and N digestibilities, despite herbage intakes being lower than intended. However, both [^{3}H]milk fat and [^{3}H]milk protein digestibilities declined only very slightly. These reductions in milk digestibility could reflect a decline in true digestibility or a change in the extent of ^{3}H recycling into the digestive tract. Unexpectedly, in the first period, the [^{3}H]milk protein and total dietary N digestibilities were significantly ($P<0·05$) different, although the two fat digestibility estimates were similar.

The results suggest that there is a small reduction in milk digestibility with advancing age and increasing herbage intake.

Studies on the kinetics of dietary zinc absorption in the rat. By DAPHNE E. COPPEN, N. T. DAVIES and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It has been shown that ^{65}Zinc absorption in vivo from duodenal ligated loops exhibited saturation kinetics indicative of a carrier-mediated process (Davies, 1980). To investigate the kinetics of absorption of dietary Zn, groups of weanling rats were trained to a meal-feeding regimen and received a semisynthetic diet containing 40 mg Zn/kg for 14 d. On the 15th day, they were offered test meals containing 5, 10, 20, 40, 80 and 160 mg Zn/kg each labelled with ^{65}Zn. An additional group of rats received an intraperitoneal injection of ^{65}Zn and was offered a meal containing no radioactive Zn. The decline in whole-body ^{65}Zn was monitored over an 8-d period.

Plots of the log of ^{65}Zn retention against time were biphasic; the first phase representing the loss of unabsorbed ^{65}Zn in the faeces over the initial 72 h, while the second linear phase represented the biological turnover of ^{65}Zn. The true fraction of ^{65}Zn absorbed was derived from extrapolation of this second exponent back to zero time ($Y2$) and expressed as a proportion of the same ordinate for the intraperitoneally dosed rats ($Y1$). It was apparent that the amount of Zn absorbed increased in a curvilinear fashion towards a maximum as dietary Zn increased. From a double reciprocal plot of the amount of Zn absorbed against soluble Zn concentration in the gut lumen (estimated from soluble Zn concentrations in gastric contents at slaughter) a Michaelis–Menten constant (K_m) of 95·4 μg/ml was derived; this value agrees with that obtained previously using ligated loops (Davies, 1980).

To investigate Zn homoeostatic control further, groups of weanling rats were maintained on a semisynthetic diet; the regimen was as previously described. The diets offered contained 5, 10, 20, 40, 80 and 160 mg Zn/kg. On the 15th day, the rats were offered a single test meal containing ^{65}Zn and 20 mg Zn/kg and then maintained on their previous diets for a further 8 d. During this period, whole-body ^{65}Zn retention was monitored as above. The results showed that when dietary Zn concentration was between 5 and 80 mg/kg, homoeostatic regulation was effected by modulation of both absorption and excretion. However, when dietary Zn contents were in excess of 80 mg Zn/kg, Zn homoeostasis was achieved solely by changes in Zn excretion.

Davies, N. T. (1980). *Br. J. Nutr.* **43**, 189.

Leucocyte and tissue zinc concentrations in the growing pig. By R. W. CROFTON,* M. CLAPHAM,* W. R. HUMPHRIES,† P. J. AGGETT* and C. F. MILLS,† **Department of Medicine, University of Aberdeen, Aberdeen* and *†Department of Nutritional Biochemistry, Rowett Institute, Bucksburn, Aberdeen*

Plasma zinc concentration is a poor indicator of the adequacy of Zn nutriture; leucocyte Zn concentration has been suggested as a better guide, the advantage of leucocytes being that they are readily accessible. This study aimed to measure and compare leucocyte and tissue Zn concentrations in the growing pig consuming Zn-adequate or Zn-deficient diets.

Twenty-seven uncastrated boars were randomly allocated into three groups at 8 weeks of age. One group was offered a maize/soya-bean diet high in phytate and calcium and low in Zn content; a second group was pair-fed with a Zn-supplemented (100 mg/kg) diet and the third group was offered the latter diet *ad lib.* Three weeks after starting on the diet one animal from each group was slaughtered each week. Plasma and leucocytes were separated from blood taken at slaughter and tissue samples obtained from various sites.

All Zn-deficient animals showed signs of parakeratosis at slaughter and their rate of weight gain was 0·48 (SE 0·31) kg/d compared with 0·73 (SE 0·27) kg/d for the animals offered the Zn-supplemented diet *ad lib.* ($P<0·001$). In contrast to evidence obtained in human studies (Jones *et al.* 1981), the Zn content of pig leucocytes was not significantly correlated to that of muscle (n 13; r 0·52). The Zn content of plasma, liver and pancreas (Table) was significantly depressed during Zn deficiency. Zn in leucocytes and muscles was not influenced by the above diets and it is concluded that the Zn content of these tissues does not provide a reliable indicator of the adequacy of the Zn status of the growing pig.

Plasma and tissue zinc

Group	Dietary Zn (mg/kg)	Plasma (μmol/l) Mean	Plasma SD	Leucocyte (μmol/kg DM) Mean	Leucocyte SD	Liver (mmol/kg DM) Mean	Liver SD	Pancreas (mmol/kg DM) Mean	Pancreas SD	Abdominal muscle* (mmol/kg DM) Mean	Abdominal muscle* SD
1. *Ad lib.*	140	21·9	4·6	328·2	160·0	2·13	0·43	2·47	0·70	2·18	0·70
2. Pair-fed	140	19·5	3·0	371·7	174·5	2·62	0·89	2·95	1·11	2·64	0·91
3. Zn-deficient	40	4·9	2·0	382·1	71·2	1·48	0·19	1·54	0·13	2·42	0·84
Levels of probability (P)											
1 *v.* 3		<0·001		NS		<0·025		<0·025		NS	
2 *v.* 3		<0·001		NS		<0·001		<0·0025		NS	

*Representative of four different muscles.
NS, not significant.

P.J.A. thanks the Rank Prize Fund for financial support.

Jones, R. B., Keeling, P. W. N., Hilton, P. J. & Thompson, R. P. H. (1981). *Clin. Sci.* **60**, 237.

The excretion of N^{τ}-methyl histidine by cockerels. By C. I. HARRIS, G. MILNE and R. M. MCDIARMID, *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The excretion of endogenous N^{τ}-methyl histidine (N^{τ}-MH) has been proposed as a measure of muscle protein breakdown in certain mammals (Harris & Milne, 1981) where the criterion of validity is the rapid, quantitative recovery in urine of labelled N^{τ}-MH administered intravenously. Studies of N^{τ}-MH excretion and metabolism have not been made in birds, apart from the comparisons of normal and dystrophic fowl by Hillgartner *et al.* (1981) who did not attempt any validation of the above type.

Cockerels (Ross I broiler chicken, Ross Poultry (GB) Ltd, Inverurie), were purchased as day-old chicks and given a cereal-based diet devoid of fish meal and meat products. Excreta were collected daily in 0·25 M-HCl following subcutaneous injection of N^{τ}-[$^{14}CH_3$]-MH (3–6 μCi/kg body-weight) and the supernatants from the homogenized excreta were measured both for total radioactivity recovered and the distribution of radioactivity (Harris & Milne, 1981).

Mean recoveries of radioactivity in excreta from groups of three cockerels

	Percentage of injected dose					
Days . . .	1	2	3	4	Total	SD
Age (weeks)						
6	76·6	9·1	2·3	1·3	88·7	5·8
9	59·6	14·6	4·9	2·1	81·1	6·0
12	37·4	12·3	9·1	3·4	60·2	4·1
18	35·3	20·5	9·3	5·2	70·4	1·9

The recovery of radioactivity progressively decreased with age, suggesting that excreted N^{τ}-MH was not an adequate index of muscle protein breakdown in older birds. The distribution of radioactivity showed that excreted N^{τ}-MH was only slightly degraded (<5%) in the youngest birds although a progressive increase in degradation was found with increasing age. Total N^{τ}-MH excretion decreased from 13·4 μmol/kg body-weight per d at 6 weeks (mean live weight 1208 g) to 11·6 μmol/kg body-weight per d at 18 weeks (mean live weight 4583 g).

Harris, C. I. & Milne, G. (1981). *Br. J. Nutr.* **45**, 411.
Hillgartner, F. B., Williams, A. S., Flanders, J. A., Morin, D. & Hansen, R. J. (1981). *Biochem. J.* **196**, 591.

Apparent re-esterification of fatty acids during lipolysis in pregnant ewes.

By S. WILSON, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY*

In vitro experiments have suggested that during lipolysis re-esterification of fatty acids can occur within the adipose tissue of ruminants (Vernon, 1980). As part of a study to describe the short-term changes that occur in adipose tissue metabolism in pregnant ewes, the extent of apparent re-esterification of fatty acids was determined in vivo by comparing the entry rates of glycerol and free fatty acids (FFA) into the peripheral circulation.

Six monotocous Scottish Blackface ewes of similar total body fat content (approximately 10 kg) were fed on either diet A (700 g dried grass + 100 g hay/d; ether extract, 20 g) or diet B (1500 g dried grass + 100 g hay/d; ether extract, 40 g) by continuous feeder from day 100 of gestation. On days 120 and 123 of gestation they were given continuous intrajugular infusions (5 h) of [2-^{3}H]glycerol (1 μCi/2 μmol per min) and [9,10(*n*)-^{3}H]palmitate (1 μCi/0·6 μmol per min, prepared as described by Lindsay & Leat, 1977) respectively. The plateau specific radioactivities of glycerol and total FFA were determined from five plasma samples taken during the last hour of infusion. The measured estimates of FFA entry rate, determined with [9,10(*n*)-^{3}H]palmitate, are compared in the Table with the derived estimates, determined from the glycerol entry rates (assuming 3 mol FFA released per mol glycerol).

	Glycerol entry rate (g/d)		FFA entry rate (g/d)				Apparent re-esterification (g/d)	
			[9,10(*n*)-^{3}H]palmitate		[2-^{3}H]glycerol			
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	64	7·3	268	21·1	570	65·3	302	52·7
B	55	3·8	162	11·6	493	34·2	331	31·1

FFA entry rates determined with [9,10(*n*)-^{3}H]palmitate were approximately 65% higher ($P<0·01$) in the ewes fed on diet A than in those fed on diet B. For both diets, estimates of FFA entry rate determined with [2-^{3}H]glycerol were much higher ($P<0·001$) than the estimates determined with [9,10(*n*)-^{3}H]palmitate. Thus, even in the ewes fed on diet A, which resulted in a state of moderate undernourishment (plasma 3-hydroxybutyrate concentrations of 1·6 (SE 0·48) mM), large amounts of fatty acids appeared to be re-esterified within the adipose tissue.

As glucose is considered to be the major precursor of glycerol-3-phosphate required for the esterification of fatty acids to triacylglycerols in sheep adipose tissue (Vernon, 1980), approximately 18 (SE 1·4) g glucose could have been used for re-esterification in the present experiments. This would account for approximately 15% of daily glucose production in monotocous ewes fed on a similar diet in late pregnancy (Wilson, 1982).

Lindsay, D. B. & Leat, W. M. F. (1977). *J. agric. Sci., Camb.* **89**, 215.
Vernon, R. G. (1980). *Prog. Lipid Res.* **19**, 23.
Wilson, S. (1982). PhD Thesis, University of Nottingham.

Adenosine and metabolism of sheep adipose tissue during lactation.

By R. G. VERNON, E. FINLEY and ELEANOR TAYLOR, *Hannah Research Institute, Ayr KA6 5HL, Scotland*

The composition of milk fat depends not only on the diet of the animal but also on the contribution of adipose tissue lipid, which can be substantial (Bauman & Currie, 1980). The control of adipose tissue metabolism during lactation is not fully understood. Preliminary studies with rats have shown, rather surprisingly, that the lipolytic-response of fat cells to noradrenaline is reduced during lactation (Aitchison *et al.* 1982), due to an increased sensitivity of the fat cells from lactating rats to the antagonistic effects of adenosine (R. G. Vernon, unpublished results), a local antilipolytic agent (Fain, 1980).

Adenosine is synthesized at the cell surface from AMP by 5′-nucleotidase, an ectocellular enzyme, and is thought to exert its effects on adipose tissue metabolism via specific receptors on the fat cell plasma membrane (Fain, 1980).

5′-Nucleotidase (*EC* 3.1.3.5) activity was measured in homogenates of ovine subcutaneous adipose tissue essentially as described by Green & Newsholme (1981). The activity of the enzyme per mg DNA showed seasonal variation in control, unmated ewes, rising to a peak in May and falling to 84 (SE 14) nmol/min per mg DNA by October, paralleling changes in the capacity of the tissue for fat synthesis (results not shown).

5′-Nucleotidase activity of ovine subcutaneous adipose tissue (nmol/min per mg DNA)

State ...		Pregnant (d)				Lactating (d)			
		85 January		135 March		15 April		45 May	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pregnant/lactating	7	71	21	74	13	51	10	62	15
Control (unmated)	5	50	23	57	6	114	32	166	48

The seasonal surge in 5′-nucleotidase activity was not observed in lactating sheep. The mean cell volume of fat cells from lactating sheep fell by more than 50% between day 135 of pregnancy and day 45 of lactation, indicating a considerable mobilization of lipid. In addition, the capacity to synthesize fat is known to be suppressed in sheep adipose tissue during lactation (Vernon *et al.* 1981). These observations, although not conclusive, suggest that adenosine has an important role in the regulation of adipose tissue metabolism in sheep.

Aitchison, R. E. D., Clegg, R. A. & Vernon, R. G. (1982). *Biochem. J.* **202**, 243.
Bauman, D. E. & Currie, W. B. (1980). *J. Dairy Sci.* **63**, 1514.
Fain, J. N. (1980). In *Biochemical Actions of Hormones*, vol. 7, p. 119 [G. Litwack, editor]. London and New York: Academic Press.
Green, A. & Newsholme, E. A. (1981). *Biochim. biophys. Acta* **676**, 122.
Vernon, R. G., Clegg, R. A. & Flint, D. J. (1981). *Biochem. J.* **200**, 307.

Attempts at improving the estimation of ruminal volatile fatty acid production rate in sheep. By R. W. MAYES, C. S. LAMB and P. M. COLGROVE, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 oPY*

In the estimation of ruminal volatile fatty acid (VFA) production rates in continuously-fed sheep, receiving 18-h isotope infusions, considerable variability in VFA specific radioactivity (SRA) measurements within sheep has sometimes been observed. Three experiments with fistulated sheep given hay and concentrate diets by continuous feeder were carried out to attempt to reduce this variability either by incorporation of isotope into the diet, or by increasing the duration of isotope administration, or by using samples of digesta from the abomasum.

In the first experiment, two sheep, on separate occasions, received [1-^{14}C]butyrate for 18 h, either by infusion (I) or in the diet absorbed into shredded paper (F). Within each sheep, coefficients of variation (CV, %) of butyrate SRA in six rumen-liquor samples, taken over the last 5 h of each period of administration, were lower when the isotope was given by F (33·5) than by I (92·8). Method F gave higher estimates of butyrate irreversible loss rate (ILR) than method I. When similar comparisons were made in a second experiment using eleven sheep and [U-^{14}C]acetate, differences between F and I in CV of acetate SRA were smaller than found previously (F, 25·5; I, 26·4; SE 4·2). Significantly higher ($P<0·01$) acetate ILR values were obtained with F (134 g C/d) than with I (96 (SE 6·2) g C/d).

In a third experiment in which ten sheep were given [U-^{14}C]acetate by methods I or F for 54 h, the CV of acetate SRA and estimates of acetate ILR from six hourly rumen-liquor samples taken after 36 h were not significantly different from samples taken after 12 h. Values of CV of acetate SRA in six abomasal digesta samples taken over the final 24 h of the experiment were significantly ($P<0·01$) lower (15·6) than values obtained from rumen liquor after 36 h (43·4, SE 4·2); acetate ILR estimates from abomasal samples were significantly ($P<0·001$) higher than rumen ILR values. From sheep given the isotope by method F, samples of rumen liquor taken by stomach tube gave CV of acetate SRA and acetate ILR estimates which were not significantly different from values obtained from samples taken via the rumen fistula.

The results of these experiments suggest that incorporation of an isotope in the diet may be used as an alternative to ruminal infusion when making VFA production-rate estimates. There was no benefit in extending the period of isotope administration, except if abomasal digesta were used and the reasons for high ILR estimates can be established. Estimation of ruminal VFA production rate in intact sheep is possible by dietary incorporation of isotope and the use of the stomach tube to obtain rumen samples.