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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Ninetieth Meeting of the Nutrition Society (One Hundred and Fifty-fourth of the Scottish Group) was held in the School of Agriculture, Aberdeen, on Thursday and Friday, 15/16 *September* 1983, *when the following papers were read:*

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The use of the rumen simulation technique (Rusitec) to investigate factors limiting the rate of digestion of plant cell walls by a mixed population of rumen micro-organisms. By R. E. BRICE and I. M. MORRISON, *Hannah Research Institute, Ayr KA6 5HL*

Brice & Morrison (1983) found that when hay stem, extracted to remove soluble constituents, was incubated in nylon bags in an artificial rumen, Rusitec (Czerkawski & Breckenridge, 1977), the extent of digestion varied with the pore size of the nylon bags. Over a 48 h period, dry matter (DM) loss from 10 mm-chopped stem from the larger pore bags (pore size range 24–1000 μm, DM loss 28–32%) was significantly greater than the DM loss (10%) from the 5 μm bag. Furthermore, whilst there was loss of xylose residues from the cell wall material in the larger pore size bags there was virtually no loss of xylose from the 5 μm bag over this period. To investigate this observation further, the loss of xylose and glucose residues (as indices of hemicellulose and cellulose digestion respectively) from 10 mm-chopped stem in 1000 or 5 μm bags was followed over a 72 h period. Milled stem (passed through a 1 mm screen) was also incubated in 5 μm bags to determine whether an increase in the surface area of cell wall to enzymic degradation would change the pattern of cell wall digestion. The % decreases in the amounts of the xylose and glucose residues are given in the Table.

Bag pore size (μm)	Stem treatment	Time (h)...	Xylose residues			Glucose residues		
			24	48	72	24	48	72
1000	Chopped		18·9	37·6	43·4	15·9	38·0	46·7
5	Chopped		0	2·1	9·8	0	5·1	16·6
5	Milled		0·6	20·8	29·6	3·7	20·0	36·4

The digestion of both hemicellulose and cellulose was reduced when the 10 mm-chopped stem was incubated in the 5 μm bags but this effect was offset when the samples were milled. It is concluded that the 5 μm pore size restricts the inflow into the bag of a component of the microbial enzyme system concerned with cell wall digestion. This component appears to have a role in the initial disruption of the assemblage of the cell wall constituents, exposing hemicellulose and cellulose to attack by xylanases and cellulases.

Brice, R. E. & Morrison, I. M. (1983). *Proceedings of the Nutrition Society* **42**, 30A.
Czerkawski, J. W. & Breckenridge, G. (1977). *British Journal of Nutrition* **38**, 371–384.

Studies of rumen microbial protein synthesis using ^{32}P incorporation in a model in vitro system. By R. J. MERRY, R. H. SMITH and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

An in vitro continuous culture system designed to model rumen processes has been described by Merry *et al.* (1983*a*). Solid feed is added continuously to a culture vessel and liquid and solid turnover rates can be varied independently. The approach allows simulation of the rumen environment so that studies of microbial protein synthesis and dietary protein degradation can be carried out under steady-state conditions. Two culture vessels were used (1130 ml capacity) and fed with pelleted diets. Diet 1 provided (g/d) straw 16·9, barley 8·9, tapioca 6·6 and urea 0·7. Diet 2 provided (g/d) straw 16·3, barley 8·6, tapioca 2·7, fish meal 5·3 and urea 0·3. Both diets supplied 32 g organic matter/d and approximately 35 g rumen degradable nitrogen/kg organic matter truly digested and adequate sulphur and phosphorus to satisfy rumen microbial requirements. Liquid (artificial saliva) entry rate was 69 ml/h. Approximately 31 ml/h were removed as a filtered effluent containing only 6 g suspended solid matter/l. The remainder emerged by overflow and contained 19 g suspended solid matter/l. ^{32}P-labelled phosphate (approximately 80 μCi/d) was infused with the artificial saliva from day 4. At 24 h intervals, amounts of ^{32}P flowing out of the vessels bound in nucleic acid were estimated (Merry *et al.* 1983*b*) and microbial N synthesis calculated from the flow and the nucleic acid-^{32}P:N value in bacterial samples harvested from the culture vessels at the end of the experiment. Approximately steady-state conditions were achieved after 4 d of ^{32}P infusion. Results (means of 5 daily values) are presented in the Table.

	Diet 1				Diet 2			
Sampling period (d) . . .	8–12		15–19		8–12		15–19	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Organic matter truly digested (OMTD) (g/d)	17·8	0·8	16·0	0·5	15·6	0·4	15·7	0·6
Non-ammonia-N outflow (g/d)	0·69	0·01	0·72	0·02	0·86	0·02	0·86	0·02
Microbial N outflow (g/d)	0·48	0·02	0·45	0·01	0·45	0·02	0·45	0·01
Microbial N synthesized/kg OMTD	27·0	1·2	28·2	1·0	29·0	1·4	28·7	1·6
Microbial N/non-ammonia-N in outflow	0·70	0·03	0·63	0·02	0·52	0·01	0·52	0·02
pH	6·30	0.09	6·35	0·06	6·67	0·04	6·70	0·05
Ammonia (mmol/l)	6·2	0·4	6·3	0·3	11·2	0·8	11·8	0·2

Stable conditions were achieved in 8 d and maintained until 19 d. The results, which have been confirmed in a second experiment, showed that microbial activity within the vessels led to processes similar to those commonly found in the rumen in vivo. No differences in microbial N flow or organic matter digestion were apparent between the two diets, but there were greater flows of ammonia and residual dietary protein with diet 2 than with diet 1.

Merry, R. J., Smith, R. H. & McAllan, A. B. (1983*a*). *Proceedings of the 4th International Symposium on Protein Metabolism and Nutrition,* Clermont Ferrand, September 1983. (In the Press.)

Merry, R. J., Smith, R. H. & McAllan, A. B. (1983*b*). *British Journal of Nutrition* **48**, 287–304.

A comparison of bacterial fermentation end-products in carnivores, herbivores and primates including man. By LINDA F. MCKAY and M. A. EASTWOOD, *Wolfson Gastrointestinal Laboratories, University Department of Medicine, Western General Hospital, Edinburgh EH4 2XU*

Anaerobic bacteria in the rumen and large intestine of monogastric animals produce methane, hydrogen, short-chain fatty acids (SCFA) and carbon dioxide. Hydrogen and methane are excreted in breath, flatus and during eructation. It is possible that methane-producing bacteria are present in herbivores and some omnivores to allow for the salvage of energy from dietary fibre in the colon. We have studied fresh faeces from different carnivores, herbivores and primates including man. Details of diet were obtained for each species. A measure of bacterial mass (diaminopimelic acid) (Czerkawski, 1974), bacterial metabolic activity, faecal SCFA (Spiller *et al.* 1980) and the ability of faecal microflora to produce hydrogen and methane (McKay *et al.* 1982) while incubated at 37° in phosphate-buffered saline for 24 h were investigated. Results for SCFA are shown in the Table.

Species (*n* 3)	Concentration of SCFA (mg/g)	SCFA molecular proportions					
		Acetic	Propionic	Iso-butyric	Butyric	Isovaleric	Valeric
Hyena	29·6	44	32	2	17	3	2
Panther	14·2	41	35	2	19	3	0
Tiger	21·2	45	34	2	16	3	0
Brown bear	17·8	76	4	1	19	0	0
Camel	4·4	50	28	6	13	2	1
Duiker	6·8	69	22	2	5	2	0
Hippopotamus	13·3	78	11	2	6	3	0
Oryx	11·4	79	15	0	6	0	0
Diana monkey	10·6	71	16	0	11	1	1
Chimpanzee	19·4	75	14	0	8	1	2
Stumptail macaque	17·5	79	12	0	8	1	0
Orangutan	16·3	66	17	0	13	2	2
Homo sapiens (*n* 12)	22·2	53	17	2	20	4	4

The concentrations of human faecal SCFA were consistently closer to those of carnivores and may reflect the high proportion of animal protein and fat and low levels of cereals in the diet.

Faecal incubation yielded headspace hydrogen concentrations (herbivores > man > carnivores > primates) and methane concentrations (herbivores > primates > man). No methane was detected from carnivores. Faecal bacterial mass was related to headspace methane concentrations (r 0·77, $P<0·01$) and total headspace gas (r 0·609, $P<0·05$). This study demonstrates differences in bacterial fementation end-products between carnivores, herbivores and primates and suggests bacterial adaptations to diet.

We are grateful to the Royal Zoological Society, Edinburgh for their assistance.

Czerkawski, J. W. (1974). *Journal of the Science of Food and Agriculture* **25**, 45–55.
McKay, L. F., Holbrook, W. P. & Eastwood, M. A. (1982). *Acta Pathologia, Microbiologica et Immunologica Scandinavica* **90**, Sect. B, 257–260.
Spiller, G. A., Chernoff, M. C., Hill, R. A., Gates, J. E., Nasser, J. J. & Shipley, E. A. (1980). *American Journal of Clinical Nutrition* **33**, 754–759.

Regulation of pyruvate kinase activity and glucose utilization by insulin in sheep adipose tissue maintained in tissue culture. By R. G. VERNON and ELEANOR TAYLOR, *Hannah Research Institute, Ayr KA6 5HL*

Sheep adipose tissue remains metabolically active for several days when maintained in tissue culture (Vernon, 1979) and retains sensitivity to hormones (Vernon, 1982). Tissue culture has been used to study the regulation of glucose metabolism in sheep adipose tissue and has indicated a role for pyruvate kinase (Robertson *et al.* 1982).

Pieces of subcutaneous adipose tissue taken from 8- to 10-month-old sheep were maintained in tissue culture at 37° in Medium 199, buffered with 25 mM-Hepes, supplemented with various antibiotics (Robertson *et al.* 1982) with air:CO_2 (95:5 v/v) as gas phase. In this system the activity of pyruvate kinase (*EC* 2.7.1.40), measured using 3 mM-phosphoenol pyruvate plus 3 mM-fructose 1,6-biphosphate (Robertson *et al.* 1982) and the rate of glucose uptake from the medium were both stimulated by physiological concentrations of insulin.

Effect of insulin on glucose utilization and pyruvate kinase activity of pieces of sheep adipose tissue maintained in tissue culture for 48 h

(Mean values with standard errors for five observations)

Insulin in medium (ng/ml)	Glucose utilization (nmol/h per 10^5 cells)		Pyruvate kinase activity (μmol/min per mg protein)	
	Mean	SEM	Mean	SEM
0	33	6	0·84	0·13
1	55	7	1·01	0·17
10	65	10	1·28	0·15
100	68	9	1·58	0·30

The concentrations of insulin required for half-maximum stimulation of the processes were 4 and 0·6 ng/ml for pyruvate kinase activity and glucose uptake respectively. Preliminary studies show that insulin loss from the culture medium is low (about 10% over the first 24 h of tissue culture).

The results provide further evidence that: tissue culture provides a valid system for studying the metabolism of sheep adipose tissue; glucose utilization is modulated by insulin in sheep adipose tissue; pyruvate kinase is probably involved in the control of glucose utilization in sheep adipose tissue.

Robertson, J. P., Faulkner, A. & Vernon, R. G. (1982). *Biochemical Journal* **206**, 577–586.
Vernon, R. G. (1979). *International Journal of Biochemistry* **10**, 57–60.
Vernon, R. G. (1982). *International Journal of Biochemistry* **14**, 255–258.

Modulation of noradrenaline-stimulated lipolysis by an adenosine analog in adipose tissue from control and lactating sheep. By R. G. VERNON and E. FINLEY, *Hannah Research Institute, Ayr KA6 5HL*

Lipolysis in adipose tissue can make a substantial contribution to the production of milk-fat (Bauman & Currie, 1980). Studies with rat adipocytes have shown that the rate of noradrenaline-stimulated lipolysis is, paradoxically, inhibited to a greater extent by adenosine during lactation than pregnancy (Vernon & Flint, 1984). Preliminary studies with adipose tissue from sheep incubated with noradrenaline in Medium 199 (a medium which appears to promote adenosine production) suggested that adenosine may be limiting the rate of noradrenaline-stimulated lipolysis to a greater extent in tissue from lactating than non-lactating sheep also. The present study was designed to test this conclusion.

Pieces of subcutaneous adipose tissue, obtained from control and 20-d lactating sheep, were incubated for 3 h at 37° in Krebs–Ringer bicarbonate buffer, containing 25 mM-Hepes (pH 7·3), 5·5 mM-glucose, 1 mM-acetate and 40 mg albumin/ml; lipolysis was measured by the rate of glycerol release. Addition of 1 μM-noradrenaline resulted in a marked increase ($P<0·001$) in the rate of lipolysis (see Table) which was greater ($P<0·05$) with tissue from lactating sheep. The addition of 20 nM-N^6-phenylisopropyl adenosine (PIA), an analog of adenosine which is not metabolized by adenosine deaminase within the tissue, markedly decreased the rate of noradrenaline-stimulated lipolysis in adipocytes from both control and lactating sheep but to a significantly greater extent ($P<0·02$) in adipocytes from lactating sheep. Addition of 1 μM-PIA had no greater effect than 20 nM-PIA (results not shown).

Effect of 1 μM-*noradrenaline* (*NA*) *and* 20 nM-N^6-*phenylisopropyl adenosine* (*PIA*) *on lipolysis in adipocytes from lactating and control* (*non-lactating*) *sheep*

(Mean values with standard errors for five observations)

	nmol glycerol released/3 h per 10^5 cells							
	Basal		NA		NA + PIA		NA − (NA + PIA)	
State	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	51	18	525	73	285	95	239	57
Lactating	34	13	783	81	298	52	485	56

Lactation results in an increased response of sheep adipose tissue to the anti-lipolytic effect of adenosine (assuming it has the same effect as its analog PIA) as previously shown for the rat. The physiological significance of this mechanism is not yet clear.

Bauman, D. E. & Currie, W. B. (1980). *Journal of Dairy Science* **63**, 1514–1529.
Vernon, R. G. & Flint, D. J. (1984). In *Physiological Strategies in Lactation* [M. Peaker, C. H. Knight and R. G. Vernon, editors]. London and New York: Academic Press. (In the Press.)

Fatty acid metabolism of the perfused caudate lobe from fed and starved non-pregnant and pregnant sheep. By S. M. BUTLER, R. G. VERNON and A. FAULKNER, *Hannah Research Institute, Ayr KA6 5HL*

Multiparous ewes in the last third of pregnancy are susceptible to ketosis. The aetiology of the disorder is still poorly understood but the regulation of hepatic metabolism of fatty acids may well be different in ewes susceptible to ketosis. The regulation of lipid metabolism has been studied in in vitro preparations of rat liver by comparing the metabolism of long and medium chain fatty acids: medium chain, unlike long chain fatty acids, are not esterified to any appreciable extent and their transfer into the mitochondria is not dependent on carnitine (Williamson, 1979).

The perfused caudate lobe was chosen as a model in which the metabolism of medium and long chain fatty acids could be compared in sheep liver. Lobes were obtained from starved (72 h); pregnant (124–139 d gestation); fed, non-pregnant; and starved (72 h), non-pregnant ewes. Each lobe was perfused in a re-cycling system with Krebs–Ringer bicarbonate buffer (gassed with O_2:CO_2, 95:5 v/v) containing physiological concentrations of albumin, glucose, glycerol and propionate plus [U-^{14}C]palmitate and then [1-^{14}C]octanoate at two different concentrations. ^{14}C incorporation into perfusate ketones, tissue triglycerides and perfusate CO_2 were measured. Ketogenesis from exogenous and endogenous sources was evaluated.

Total ketone production rates were significantly higher during all perfusions of lobes from starved, pregnant sheep (see Table): this difference was still apparant when ketogenesis from endogenous sources was deducted. In contrast, the lowest rates of esterification were found in lobes from starved, pregnant sheep.

Rate of total ketone production (μ*mol*/30 *min per lobe*) *during perfusion with palmitate* (*Pal*) *or octanoate* (*Oct*)

		Pal (0·4 mM)		Pal (1·2 mM)		Oct (0·4 mM)		Oct (1·2 mM)	
State	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pregnant, starved	5	95	14	122	26	93	19	128	32
Non-pregnant, fed	4	5**	2	20*	4	26*	6	37*	12
Non-pregnant, starved	5	19**	5	49*	13	35*	8	52	16

Significant differences with respect to pregnant, starved value: *$P<0.05$, **$P<0.01$.

The results suggest that exogenous fatty acids are preferentially converted to ketones in the livers of starved, pregnant sheep. In addition, the higher ketogenicity of octanoate in lobes from starved, pregnant sheep suggest a change in the intramitochondrial regulation of fatty-acyl CoA disposal.

Williamson, D. H. (1979). *Biochemical Society Transactions* **7**, 1313–1321.

The possible use of n-alkanes in herbage as indigestible faecal markers.

By R. W. MAYES and C. S. LAMB, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY*

Most plants contain hydrocarbons in the cuticular wax, with n-alkanes having odd-numbered carbon chains in the range C_{25}–C_{35} predominating (Hawke, 1973). Long-chain fatty acids in herbage (C_{19}–C_{32}) have been suggested as indigestible faecal markers for estimation of herbage intake in grazing sheep (Grace & Body, 1981). However, the n-alkanes of herbage may be more suitable as markers since they are more chemically inert and more easily analysed than long-chain fatty acids.

The faecal recoveries relative to intake of the main hydrocarbons of freshly cut ryegrass/white clover herbage were determined with five 17-week-old male lambs offered the herbage *ad lib.* Throughout a 5-d total faeces collection and for the preceding 5 d, ^{103}Ru-phenanthroline marker was given as a drench twice daily. The n-alkane contents of freeze-dried herbage and faeces were determined by gas chromatography after initial Soxhlet extraction with petroleum ether (b.p. 60–80°) and passage of the nonsaponified hexane extract through a silica gel column. Dotriacontane (C_{32} n-alkane) was added as an internal standard during the Soxhlet extraction. The digestibility of the dry matter (DM) of herbage was 0·736 (SEM, 0·0019). Concentrations of the predominant n-alkanes in herbage (mean of six daily cuts) and faeces and the proportions of that ingested recovered in the faeces (means of five lambs) are given in the Table.

n-Alkanes (carbon chain length) . . .	25		27		29		31		33		35	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Herbage content (mg/kg DM)	49	17·2	38	12·6	63	3·8	108	9·5	82	7·0	12	1·2
Faecal content (mg/kg DM)	39	17·4	61	9·6	175	28·3	344	51·5	283	32·0	45	4·6
Proportion recovered in faeces												
Mean	0·195		0·447		0·722		0·831		0·909		0·975	
Range	0·080–0·311		0·321–0·571		0·679–0·794		0·780–0·862		0·866–0·953		0·901–1·033	

There was a progressive increase in the proportion of ingested n-alkane recovered in the faeces as the chain length increased. The recovery of the C_{35} n-alkane compared favourably with that of ^{103}Ru-phenanthroline (mean, 1·018; range, 0·931–1·123).

These preliminary results suggest that the longer-chain n-alkanes in herbage could be useful as indigestible markers to determine herbage digestibility and intake in grazing animals. Further work is necessary to establish whether such factors as age and species of animal, maturity and botanical composition of the herbage and supplementary feeding can affect the faecal recovery of the n-alkanes.

Grace, N. D. & Body, D. R. (1981). *Journal of Agricultural Science, Cambridge* **97**, 743–745.

Hawke, J. C. (1973). In *Chemistry and Biochemistry of Herbage*, vol. 1, pp. 213–263 [G. W. Butler and R. W. Bailey, editors]. London: Academic Press.

Calculating standard errors for flow rates in an open-compartment model with continuous isotope infusion. By HELEN K. SMITH, *ARC Unit of Statistics, The James Clerk Maxwell Building, Edinburgh EH9 3JZ* and J. A. MILNE and R. W. MAYES, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PY*

In many nutritional studies, isotope dilution experiments with continuous infusion are used to estimate flow rates of metabolites using open-compartment models. Nolan *et al.* (1976) gave the derivation of a series of simultaneous equations for estimating flow rates from specific activities (SAs). To decide how many samples of each compartment should be taken per animal, and to consider the accuracy of the flow rates estimated, a within-animal standard error is required.

Since it is difficult to calculate standard errors for the flow rates directly from SA variances, the jack-knife method (Bissell & Ferguson, 1975) of estimating standard errors was examined. For n samples of each SA taken during the period of infusion, the data are divided into n separate sets of SAs using one sample from each compartment for each infusion. Flow rates (f_n) are calculated from the mean of all n SAs. Each set of data is excluded in turn and flow rates are calculated from the mean of the remaining (n-1) SAs (f_{-i}). Sets of flow rates are then estimated by

$$f_i = nf_n - (n\text{-}1)f_{-i}\ (i = 1 \ldots n).$$

These have mean $\bar{f}_i$ and variance W. The mean is the jack-knife flow rate estimate. The jack-knife standard error is $\sqrt{W/n}$.

Simulation was used to check the method, using data generated for 200 experiments with six samples of each SA for each infusion. The SAs had normal or log normal distributions independent of one another. For all flow rates the mean of the jack-knife standard errors was close to the true value (difference less than 10%), which indicated that when jack-knife standard errors are combined for several animals they will give reliable values.

To compare between-animal and within-animal variances, three data sets were used from experiments with sheep given infusions of ^{14}C-labelled tracers of (1) acetate, propionate and butyrate into the rumen, (2) carbon dioxide into the rumen and CO_2 and urea into blood, and (3) glucose and CO_2 into blood. The ratios of between-sheep:within-sheep variance components (B:W) found for (1) were generally between 0·01 and 5·0, for (2) between 0·2 and 1·5 and for (3) between 0·25 and 1·0. Since for n SA samples, the estimated between-sheep variance is ($B + W/n$), increasing n always reduces the variance but the extent of the benefit depends on (B/W). For the range $0{\cdot}25 < (B/W) < 1{\cdot}5$, which covers many of the data examined, six to twelve samples from each compartment need to be taken for each animal.

Bissell, A. F. & Ferguson, R. A. (1975). *Statistician* **24**, 79.
Nolan, J. V., Norton, B. W. & Leng, R. A. (1976). *British Journal of Nutrition* **35**, 127–147.

Response of young rapidly growing lambs to trenbolone acetate combined with oestradiol-17ß. By S. B. SINGH, H. GALBRAITH, G. D. HENDERSON and GAIL FORBES, *School of Agriculture, 581 King Street, Aberdeen, Scotland AB9 1UD*

The combination of trenbolone acetate (TBA) and oestradiol-17β (OE_2) is effective in stimulating growth in certain animals (Galbraith & Topps, 1981). The work reported here investigated aspects of the response of young, rapidly growing lambs to this combination.

Ten castrate male lambs, aged about 2 months and weighing 25 kg, were either sham-implanted controls or implanted with 35 mg TBA + 5 mg OE_2 ('Revalor', Hoechst) 42 d before slaughter. They were offered a commercial fattening diet with a metabolizable energy content of 12·8 MJ/kg dry matter (DM) restricted to 90% of estimated voluntary DM intake (DMI). Results are shown in the Table. Steroid-treated lambs gained more weight and had heavier empty body-weights (EBW) and carcass weights (CW) than the controls. Chemical analysis of the minced carcass was carried out but there was no significant difference between the groups in the contents of crude protein, fat and ash (other results are given in the Table).

	Gain (g/d)	DMI (kg/d)	EBW (kg)	CW (kg)	TG (g/kg EBW)	KCF (g/kg EBW)	Retained N (days 4–7) (g/d)
Control	375	1·08	33·6	19·4	0·112	5·58	15·4
TBA + OB_2	424	1·14	35·0	20·0	0·08	7·64	18·7
SED	30·2	0·063	2·0	0·64	0·013*	0·83*	0·8**

*$P<0·05$, **$P<0·01$.

Treated lambs had lighter thyroid glands (TG, $P<0·05$) and a greater content of kidney + channel fat (KCF) than controls. A nitrogen-balance study was conducted for 6 d before and 7 d after, implantation. The results showed that steroid-treated lambs retained more N than the controls ($P<0·05$) as early as days 4–7 after implantation. The response of the lambs to adrenocorticotrophic hormone (ACTH) (10 i.u. ACTHAR given by injection, commencing at 10.00 hours) was studied for a 24 h period, 28 d after implantation. Plasma cortisol concentrations in steroid-treated lambs were less than those in the controls (35·3 *v.* 50·3 nmol/l) for 3 h after ACTH injection. Plasma glucose concentrations were also less (4·83 *v.* 5·58 mmol/l) during the 24 h period. The apparent suppression of adrenal activity may be similar to that observed by Thomas & Rodway (1982) in female sheep treated with TBA.

Galbraith, H. & Topps, J. H. (1981). *Nutrition Abstracts and Reviews, Series B* **51**, 521–540.

Thomas, K. M. & Rodway, R. G. (1982). *Proceedings of the Nutrition Society* **41**, 138A.

Response of the female rat to trenbolone acetate and oestradiol-17β given alone or combined. By H. GALBRAITH and PAULINE A. LAWRIE, *School of Agriculture, 581 King Street, Aberdeen, Scotland AB9 1UD*

Trenbolone acetate (TBA) and oestradiol-17β (OE_2) are used alone or in combination to promote growth in ruminant animals (Galbraith & Topps, 1981). The work reported here investigated the effect of these compounds in the female rat, an animal used previously for studies on mode of action (Vernon & Buttery, 1978).

Forty female rats (mean live weight 115 g) were allocated to receive by injection, on days 1, 3 and 5 of each week, for an average of 30 d, one of the following treatments: vehicle only (treatments C_{al} and C_{pf}), 2 mg TBA (T_2), 4 mg TBA (T_4), 160 μg OE_2 (O) or 2 mg TBA + 160 μg OE_2 (T_2+O) /kg body-weight. All groups were fed *ad lib.* except C_{pf} which was pair-fed with group O. The results are summarized in the Table.

	C_{al}	T_2	T_4	C_{pf}	O	T_2+O	SEM
No. of rats ...	7	7	5	7	7	7	
Live-weight gain (g)	109·5	126·3*	128·7*	109·3	103·2	101.6†	1·97
Food intake (g)	548	581*	584*	520	538	516*†	5·11
Carcass:							
Weight (g)	108·9	114·4*	118·0*	105·9	99·1*†	104·1†	1·26
Dry matter (g/kg)	311	308	312	312	322*†	324*†	1·70
Liver‡	86·1	82·1	87·9	86·4	110·9*†	89·5†	1·68
Kidney‡	17·3	17·6	18·1	18·1	20·7*†	19·3*†	3·77
Uterus‡	3·26	2·71	2·85	3·47	5·50*†	4·56*†	0·20
Ovary (μg)	62·0	58·9	58·6	61·1	41·9*†	33·8*†	2.02

SEM, pooled standard error of the mean.
Significantly different ($P<0·05$) from: *group C_{al}, †group T_2.
‡g/kg carcass weight.

Trenbolone acetate treatment alone increased food intake (FI), live-weight gain (LWG) and carcass weight (CW) compared with controls not treated with steroids. The use of OE_2 (in group T_2+O) reduced significantly the response to TBA in FI, LWG and CW. The use of OE_2 alone also tended to reduce these characteristics compared with *ad lib.* controls. Chemical analysis of the carcasses indicated certain differences for dry matter but not crude protein and lipid. The weights (expressed per kg of carcass weight) of liver, kidney and uterus were significantly increased by treatments O and T_2+O. The combinaton of TBA and OE_2 decreased ovarian weight to a greater extent than OE_2 given alone.

These results suggest that the anabolic effect of TBA in the female rat may be largely inhibited by simultaneous treatment with OE_2.

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Plasma fibronectin changes during acute nutritional deprivation in healthy human subjects. By S. J. D. CHADWICK, A. J. W. SIM and H. A. F. DUDLEY, *Academic Surgical Unit, St Mary's Hospital Medical School, Norfolk Place, London W2*

Fibronectin is a large glycoprotein, found both in the plasma and cellular matrix. Current concepts of its function suggest that it has many roles, including opsonic activity and cellular adhesion. In the plasma form, it is a dimer of approximately 440 000 daltons. It is present in connective tissues throughout the body and although the size of in vivo production remains unknown, many types of cultured cells in vitro are known to produce fibronectin (Vaheri *et al.* 1980). Studies in obese patients (Scott *et al.* 1982) and rats (Dillon, 1982) have shown that plasma fibronectin decreases during starvation.

This study of ten healthy male subjects (mean age 21·6 years, range 19–26) describes the changes in plasma fibronectin, complement component C_3 and serum albumin during acute starvation. The studies were conducted over a 15 d period. During the first 5 d two control measurements were taken. The subjects were then allowed only tap or mineral water for 4·5 d. In the recovery period they returned to their usual diet. Plasma fibronectin and C_3 were measured by immunoelectrophoresis, serum albumin by bromocresol green dye binding and urinary ketones by keto-diastix. The results in the Table are expressed as a percentage of the control values and differences were assessed by the Wilcoxon matched pairs, signed ranks test.

Period of (d) . . .	Starvation						Recovery					
	1		3		4		1		2		5	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fibronectin	95·5	3·7	78·0•	3·0	69·4•	4·2	64·5•	2·6	72·2•	2·1	99·6	3·2
C_3	93·8	3·5	95·7	4·8	93·3	5·6	88·6•	4·5	92·5	3·7	96·7	3·0
Albumin	99·6	1·91	100·1	2·5	105·5‡	2·5	98·4	2·2	98·4	2·4	98·4	2·2

‡$P<0·02$, •$P<0·01$.

24 h urine volumes were significantly reduced from the control value (1474 (SEM 185) ml) on the last day of starvation (834 (SEM 116) ml; $P<0·01$). Urinary ketones increased in all volunteers throughout the period of starvation. Body-weight decreased steadily by 5·4 (SEM 0·3) % by starvation day 4. Serum albumin increased but plasma fibronectin decreased during acute starvation: the probable explanation is a decrease in fibronectin synthesis rate. This being so, fibronectin may have a place in the assessment of nutritional intake and/or utilization.

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Beneficial effect of corticosteroids on food intake amongst patients with advanced cancer. By T. D. WALSH[1,2,3], F. M. CHEATER[1], E. DALY[1] and G. P. JACKSON[1,4], [1]*Dept of Clinical Studies, St. Christopher's Hospice, London SE26 6DZ,* [2]*Dept of Clinical Pharmacology and* [3]*Dept of Medical Oncology, Guys Hospital, London SE1 9RT,* [4]*Dept of Dietetics and Nutrition, St. Thomas' Hospital, London SE1 7EH*

Anorexia is frequent amongst those with cancer and may be a major contributory factor to the undernutrition common in cancer patients. Corticosteroids are often used for their effects on subjective well-being, including stimulation of appetite. Examination of energy metabolism in cancer patients (Warnold *et al.* 1978) suggests that a high energy intake may be beneficial, particularly as nutritional supplementation improves the response to active therapy (Copeland *et al.* 1977). In those with advanced cancer, nutritional deficits may contribute to symptoms, e.g. weakness, and might be correctable by dietary manipulation or appetite stimulants.

A National Health Service registered dietitian assessed the voluntary dietary intake of twelve advanced cancer patients for five consecutive days using a semi-weighed technique. Energy, protein, fat, carbohydrate, iron, calcium and dietary fibre contents were calculated using standard food composition tables (Paul & Southgate, 1978). Results suggested a low energy, iron and dietary fibre and a high Ca intake. Five of the patients were receiving corticosteroids at the time of the study but use was unrelated to the length of admission or survival after the study. Corticosteroid-treated patients showed trends towards higher intakes of all nutrients except Fe or fibre (see Table). The number of subjects was small and the differences between the two groups were not statistically significant but the results suggest the use of corticosteroids may be beneficial as appetite stimulants and further studies are planned. Caution in their use is desirable as they are also employed to treat hypercalcaemia which is common in this population. The increase in dietary Ca (because of the use of milk-based foods) noted in the corticosteroid group is therefore paradoxical and may be undesirable.

Table 1. *Daily dietary intake of patients with advanced cancer*

Treatment . . .	Non-corticosteroid (*n* 7)		Corticosteroid (*n* 5)	
	Mean	SD	Mean	SD
Energy (kJ)	4668	2500	6398	1526
Protein (g)	45	23	60	17
Fat (g)	51	28	68	19
Carbohydrate (g)	120	64	180	41
Calcium (mg)	820	360	1028	315
Iron (mg)	7	4	7	2
Dietary fibre (g)	8	6	8	4

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Factors affecting plasma zinc and copper in pregnancy. By SHEENA TUTTLE, P. J. AGGETT, DORIS M. CAMPBELL and I. MACGILLIVRAY, *Department of Obstetrics and Gynaecology, University of Aberdeen, Foresterhill, Aberdeen*

Many studies have shown a fall in plasma zinc and a rise in plasma copper during pregnancy (Hambidge & Droegmueller, 1974). Zn is 70% bound to albumin and Cu is 90% bound to ceruloplasmin in the blood. A linear relationship between Zn and albumin concentration in blood has been demonstrated in the non-pregnant state (Schechter *et al.* 1976). Also, in pregnancy, there is an expansion of plasma volume. This study was designed to assess the influence of these factors in altering the plasma levels of Zn and Cu in pregnancy.

Thirty-three normal, healthy primigravidae were followed at 5-weekly intervals from their booking visit (mean 14·2 weeks) until 6 weeks post partum. At each visit, a fasting blood specimen was collected for estimation of plasma Zn, Cu, ceruloplasmin, total serum protein and albumin. At each antenatal visit, plasma volume was measured. The results are shown in the Table.

	Period of gestation (weeks)											
	Booking		20		25		30		35		Postnatal	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma volume (ml)	2918 6	441 01	3202 5	377 66	3464 5	398 02	3642 6	328 57	3793 9	448 49	—	—
Total protein (g/l)	62 07	2 53	61 23	2 13	60 29	1 90	60 33	1 63	59 05	1 32	68 26	3 92
Serum albumin (g/l)	35 94	2 63	35 22	2 23	34·58	1·47	33 89	1 78	33 55	1 71	39 31	2 62
Total albumin (g)	104 38	17 25	112 94	13 45	119 75	14 79	123 47	12 94	127 26	16 47	—	—
Plasma zinc (μmol/l)	12 41	1 28	11 38	1 57	11 04	1 07	10 57	1 98	10 66	1 47	13 79	2 27
Total zinc (μmol)	36 37	6 17	37 13	8 01	38 11	5 76	39 39	5 71	40 59	8 69	—	—
Plasma copper (μmol/l)	27 30	4 51	28 99	4 99	29 98	4·92	30·38	5·14	30 79	4 07	21 02	5 1
Ceruloplasmin (g/l)	0·525	0 109	0 534	0 110	0 580	0 109	0·594	0 095	0 575	0 116	0 410	0 118

Plasma Zn concentration was low at the booking visit and continued to fall until 35 weeks. Total serum protein and albumin levels showed a similar pattern. There was no correlation between the levels of total protein or albumin and plasma Zn at any stage of gestation. Zn and albumin concentrations were multiplied by the plasma volume and the results expressed as total Zn and total albumin mass. Both increased ($P<0·05$, $P<0·001$ respectively) between booking and 35 weeks and good correlations were obtained between them at each stage (r 0·60–0·79).

Plasma Cu concentration was high at the booking visit and continued to increase until 35 weeks gestation. Plasma ceruloplasmin level showed a similar, though not significant rise. A good correlation (r 0·5–0·85) was obtained between the two levels at every stage of gestation.

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Nitrogen turnover measurement by primed continuous [^{15}N]glycine infusions—an evaluation in surgical patients. By A. J. W. SIM, H. WARD and A. W. JOHNSON, *Academic Surgical Unit, St. Mary's Hospital Medical School, Norfolk Place, London W2* and D. HALLIDAY, *Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ*

The calculation of nitrogen turnover from the enrichment of urinary nitrogenous end-products with ^{15}N during a continuous infusion of ^{15}N-labelled amino acid requires a plateau of isotope excretion. Primed continuous infusions of [^{15}N]glycine produce early plateaux, with similar isotope enrichments to unprimed infusions (Sim *et al.* 1981).

This study investigates the ^{15}N enrichment in urinary ammonia and urea following a primed continuous infusion of [^{15}N]glycine in seven post-operative patients. The mean priming dose was 0·31 mg ^{15}N/kg and the infusion rate 0·25 mg ^{15}N/kg per 24 h. Urines were collected at 3-hourly intervals and prepared for mass spectrometric analysis by the cationic exchange resin method described by Read *et al.* (1982). Plateaux of isotope enrichment in both urea and ammonia were achieved in all patients with mean coefficients of variation for the last four 3-hourly urines of 2·1 and 3·4% respectively. All patients had an early peak of ^{15}N enrichment of ammonia, enrichment being six times greater than that of urea in the first urine sample and four times greater than that of the final ammonia plateau value. Major differences between the plateau enrichments of urea and ammonia were seen in three of the seven patients.

		Urea		Ammonia	
Patient no.	Infusion rate (mg ^{15}N/kg per 24 h)	Plateau enrichment (atom % excess)	Turnover (mg N/kg per 24 h)	Plateau enrichment (atom % excess)	Turnover (mg N/kg per 24 h)
1	0·30	0·0757	392	0·0690	430
2	0·20	0·0497	406	0·0724	279
3	0·25	0·0692	357	0·0682	362
4	0·30	0·0381	776	0·0700	423
5	0·29	0·0624	465	0·0813	357
6	0·21	0·0364	577	0·0324	647
7	0·22	0·0611	360	0·0652	337

The mean isotope enrichment in the last four 3-hourly urine collections was the same as in a complete 12–24 h urine collection (coefficient of variation 0·82 and 0·59% for urea and ammonia respectively).

These studies demonstrate that the primed continuous infusion method with a single 12–24 h urine collection can be used in post-operative patients. The discrepancy between urea and ammonia enrichments remains unexplained. Because of the findings of a peak of ammonia enrichment, which may relate to glycine and not whole body N metabolism, caution is urged in the interpretation of single dose ^{15}N methods where ammonia is the nitrogenous end-product studied.

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