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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATION

The Three Hundred and Sixty-ninth Meeting of the Nutrition Society (One Hundred and Forty-fifth of the Scottish Group) was held at the Edinburgh School of Agriculture, Kings Buildings, West Mains Road, Edinburgh on Friday, 26 *March* 1982 *when the following papers were read:*

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A comparison of the effect of yam, cassava and alfalfa based diets on cholesterol metabolism in the rat. By M. A. EASTWOOD, I. ADAMSON, and W. G. BRYDON, *Wolfson Gastrointestinal Laboratories, Department of Medicine, Western General Hospital, Creare Road South, Edinburgh EH4 2XV*

The influence of dietary fibre on cholesterol metabolism has been investigated in man (Kay & Truswell, 1976) and rat (Eastwood & Boyd, 1967). It has been suggested that the diet consumed by rural Africans is particularly effective in avoiding atheroma or by implication reducing the serum cholesterol. Two sources of dietary fibre commonly consumed as the staple diet in southern parts of Nigeria, yam and cassava and a commercial stock diet (Spratts Laboratory Animal Diet) containing alfalfa, have been fed to Wistar rats and the effects on cholesterol metabolism observed after dietary cholesterol supplementation. Skimmed milk, with supplement of 0·2% methionine was the protein source for the yam and cassava based diets. Cholesterol was dissolved in a calculated amount of corn oil and added to the mixtures.

Mature Wistar rats (five) were fed on an alfalfa based stock diet for the duration of the experiment. Serum total and HDL cholesterol were determined at the start of the experimental period in these animals and found to be (mean±SEM) 0·78±0·09 and 0·32±0·03 respectively.

Other male rats (thirty), initially on stock diet, were divided randomly into three groups and given alfalfa, yam or cassava based diets supplemented with 5 g cholesterol/kg for 23 d. On the last 3 d of the study, faeces were collected, freeze dried and analysed for bile acids and neutral sterols. At the end of the feeding period serum was analysed for HDL and total cholesterol in all animals. Animals were sacrificed, and liver cholesterol determined. The main aspects of the results are summarized in the Table.

	Yam (*n* 10) +		Cassava (*n* 10) +		Alfalfa (*n* 10) +		Alfalfa (*n* 5) −	
Added cholesterol . . .	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Serum cholesterol (total) (mg/l)	1·47	0·09^a	1·24	0·07^b	1·04	0·04^b	0·88	0·07^c
Serum cholesterol (high density lipoprotein) (mg/l)	0·45	0·02^a	0·56	0·04^b	0·33	0·02^c	0·32	0·03^c
Liver cholesterol (mg/kg)	3·9	0·4^a	8·7	0·8^{bc}	10·1	0·6^c	4·7	0·3^a
Faecal bile acids (mg/24 h)	29·5	3·2^a	54·7	7·2^b	95·0	10·8^c	18·2	4·0^a
Neutral sterols (mg/24 h)	45·5	3·3^a	71·9	6·4^b	108·7	4·4^c	31·6	5·3^a

a,b,c, Values that do not share common superscript letter across are significantly different: $P<0·05$.

In the cassava and alfalfa fed animals there is a relatively low serum cholesterol, high liver cholesterol and high faecal steroid excretion. The alfalfa and cassava diets appear to cause a shift in cholesterol from serum to liver to faeces, preventing hypercholesterolaemia by promoting loss of faecal steroids.

Kay, R. M. & Truswell, A. S. (1976). *Lancet* i, 367.
Eastwood, M. A. & Boyd, G. S. (1967). *Biochem. Biophys. Act.* **137**, 39.

Composition and nutritive value of a partly decorticated olive-pit meal. By N. J. DROULISCOS, *Department of Biology, Nuclear Research Centre, Dimocritos, Aghia Paraskevi* and P. E. ZOIOPOULOS and G. C. GEORGIADIS, *Feeding Stuff Control Laboratory, Lycovrissi, Attiki, Greece.*

Following the extraction of oil from the olive fruit, the residue is subjected to a further extraction of oil leaving a by-product which is high in fibre and lignified tissue. This by-product is highly indigestible and has practically no feeding value. Removal of the fibrous material would give a more acceptable product that could be used as an animal food. This is achieved by causing a strong draft of air to blow through the dried crushed mass of the residue. The fibrous pit particles, being lighter, are then separated leaving a product which is considerably lower in fibre content. The partly decorticated pit meal (DPM) has the following composition (g/kg; dry matter basis); crude protein (N×6·25), 215, ether extract 40, crude fibre 130, ash 89 and contains (g/kg; dry matter basis) Ca, 8 and P, 5·5. The AOAC (1970) methods were used in the analysis.

The amino acid content was measured with column chromatography in a Spinco Beckman 120-C automatic amino acid analyser and the following results were obtained: (g/16 g N): Lys 6·46, His 2·82, Arg 8·66, Asp 10·17, Thre 3·52, Ser 4·49, Glu 19·11, Pro 4·14, Gly 3·95, Ala 4·08, Val 4·45, Meth 1·52, Cyst 1·10, Iso 4·15, Leu 7·44, Tyr 3·54, Phe 4·74. Nutritional evaluation was carried out with five Hooded rats (75 g live weight) as described by Eggum (1973). Each rat was given the diet daily in a 5 d collection and 5 d preliminary period. The experimental diet was formulated by mixing olive-pit meal with a standard protein-free diet so that the final mixture would provide 15 gN/kg DM. True crude protein digestibility (0·69) biological value (0·65) and net (0·45) were measured.

The biological value of 0·65 for the olive-pit meal compares favourably with the value of 0·67 reported for soya-bean oil meal (Drouliscos & Malefaki, 1980). The product has only been tested in the rat, a monogastric animal, while the properties of this product suggest that it may be of use in the feeding of ruminants.

Association of Official Analytical Chemists (1970). *Official Methods of Analysis* 11th ed. Washington DC: Association of Official Analytical Chemists.
Drouliscos, N. J., Malefaki, V. N. (1980). *Br. J. Nutr.* **43**, 115.
Eggum, B. O. (1973). *Bereth. Forsogslab.* no. 406.

Absence of 'diet-induced thermogenesis' in growing rats kept at 29° and offered a varied diet. By H. GILLIAN BARR and K. J. MCCRACKEN, *Department of Agricultural and Food Chemistry, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*

Rats offered a varied diet (cafeteria-feeding) consume more energy than siblings with unlimited access to a stock diet (Scalafani & Springer, 1976). Some reports (Rothwell & Stock, 1979, 1980) indicate that most of the extra energy consumed is liberated as heat and does not contribute significantly to weight gain. This has been designated 'diet-induced thermogenesis'. Most experiments have been done at temperatures below the zone of thermoneutrality but Andrews & Donne (1981) reported increases in oxygen consumption of cafeteria-fed growing rats kept at 30° which appeared to support the concept of 'diet-induced thermogenesis'. In contrast it is known that adult rats tube-fed large excesses of energy deposit fat very efficiently (McCracken & McNiven, 1981) in agreement with the expected heat increment. This study was designed to compare the utilization of energy by growing rats voluntarily consuming a varied diet or force-fed a synthetic diet formulated to provide similar intakes of nutrients.

Thirty male, Sprague-Dawley rats (Charles-River, Kent), of initial weight 272 g were allocated to one of three dietary treatments: stock diet *ad lib.* (S, *n* 6), force-fed diet (F, *n* 6), varied diet *ad lib.* (V, *n* 12) or slaughtered for initial carcass composition (*n* 6). The rats were placed in individual metabolism cages in a room at 29°. After a 21 d feeding period rats were slaughtered and the homogenized carcasses were analysed for crude protein (N×6·25), fat and ash. The metabolizable energy (ME) intakes of S and F rats were determined and those of V rats were calculated from food tables (Paul & Southgate, 1978). Energy retention (ER) was calculated using factors for protein and fat. The ME intakes (kJ/d) of S, F and V were 303, 453, 392 respectively and weight gains (g/d) were 5·46, 6·87, 8·01 ($P<0.001$). The ME intakes of V rats ranged from 334 to 478 kJ/d; i.e. 10 to 60% above the mean for group S. The ER values (kJ/d) of S, F and V were 70, 178, 128 and the energy contents of carcass gain (MJ/kg) were 12·8, 26·1, 15·8 respectively. A linear regression of ER on ME (corrected for metabolic body size, $W^{0.75}$) yielded the equation ER (kJ/d per kg $W^{0.75}$) = 0·766 ME − 381 (r 0·94). Applying the pooled efficiency value to groups S, F and V yielded estimates of the maintenance requirement (kJ/kg $W^{0.75}$) of 510, 515, and 490 respectively, which were not significantly different.

These results are contrary to those of Andrews & Donne (1981) and provide firm evidence that 'diet-induced thermogenesis' does not occur when growing rats, kept at a temperature within the thermoneutral zone, voluntarily consume excess energy as a result of being offered a varied diet.

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Paul, A. A. & Southgate, D. A. T. (1978). *The Composition of Foods*. Elsevier/North-Holland Biomedical Press.
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Gum arabic metabolism in man. By A. H. MCLEAN ROSS, M. A. EASTWOOD, W. G. BRYDON, L. F. MCKAY, D. M. W. ANDERSON and J. R. ANDERSON, *Wolfson Gastrointestinal Laboratories, Department of Medicine, Western General Hospital and Department of Chemistry, University of Edinburgh*

The effect of dietary fibre on gastrointestinal function in man varies with the source (Royal College of Physicians, 1981). The differing effects are in part due to bacterial degradation using intestinal passage (Stephens & Cummings, 1980). We have studied the effect of gum arabic (GA) on intestinal function in man.

GA is a water soluble highly branched galactan which is identifiable in or when added to intestinal contents or faeces by acidic ethanolic precipitation.

Five healthy males took 25 g GA in 125 ml water for 21 d. Food intake, glucose tolerance, intestinal transit, faecal weight and contents were measured before and at the end of the treatment period.

The intakes (g/24 h; median and range) of protein (88, 76–132) fat (111, 95–180) carbohydrate (304, 178–407) and fibre (23, 12–38) were not significantly altered between the beginning and end of the study. GA was at no time detectable in faeces. The intestinal transit time (h) (originally 51, 23–83) increased in four subjects (mean increase 26 ± 9). Faecal wet weight (147, 90–245 g/24 h) dry weight, bile acids (1·18, 0·81–2·56 mmol/24 h) volatile fatty acids (13·3, 6·4–20·6 mmol/24 h) fat and neutral sterols showed no significant change.

The plasma, glucose and insulin response to 25 g glucose was always normal and was unchanged following the GA feeding period. Serum cholesterol decreased significantly (mean decrease $0 \cdot 39 \pm 0 \cdot 04$ mmol/l, $P < 0 \cdot 05$).

The breath H_2 excretion significantly increased ($P < 0 \cdot 01$) after 25 g GA ingestion, with the fasting concentration (μmol/l) pre GA $0 \cdot 14 \pm 0 \cdot 6$ post GA feeding period $0 \cdot 29 \pm 0 \cdot 08$; at 150 min pre GA 0, post GA period $0 \cdot 34 \pm 0 \cdot 10$ and at 240 min pre GA 0, post GA period $0 \cdot 64 \pm 0 \cdot 22$.

GA appears to be degraded in the human alimentary tract as a result of bacterial action. GA ingestion decreases the serum cholesterol but not faecal weight or constituents including bile acids.

Royal College of Physicians. (1981). *Medical aspects of dietary fibre.* London: Pitman Medical.

Stephens, A. M. & Cummings, J. H. (1980). *Nature, Lond.* **284**, 5753.

Proximate composition and protein quality of five varieties of grain legumes used in animal feeding. By N. J. DROULISCOS, *Department of Biology, Nuclear Research Centre, Dimocritos, Aghia Paraskevi* and P. E. ZOIOPOULOS and G. C. GEORGIADIS, *Feeding Stuff Control Laboratory, Lycovrissi, Attiki, Greece*

There is a need for improved varieties of legumes as animal feeds that are adapted to the climate of Greece and have a low production cost. The chemical composition (g/kg) and amino acid composition (g/16 g N) of the grain of five selected varieties of legumes were determined by standard AOAC (1970) methods and column chromatography respectively and are given in the Table. Based on the FAO/WHO (1973) scoring pattern, the first limiting amino acids were methionine–cystine for the field bean varieties, for peas and for chickling vetch. Valine was the first limiting for vetch peas.

	Field beans		Peas	Vetch peas	Chickling vetch
	M-10868	M-9643	(K-129)	(B_1-65)	(L-121)
Chemical composition (g/kg)					
Crude protein	259	255	235	258	220
Fat	15·3	14·3	8·9	4·0	3·8
Crude fibre	74	74	58	45	66
Ash	29	29	27	32	34
Ca	1·08	1·08	0·70	1·25	2·30
P	4·62	5·21	4·00	4·72	3·26
Amino acid composition (g/16 g N)					
Lysine	4·8	6·1	6·0	5·5	5·6
Valine	3·7	4·4	3·7	1·7	4·7
Methione-cystine	1·8	1·8	1·8	2·1	1·9
TD	0·91	0·92	0·79	0·89	0·89
BV	0·60	0·57	0·52	0·53	0·65
NPU	0·55	0·52	0·41	0·47	0·53

The protein quality of these varieties of grain legumes was measured using Hooded Listar rats as described by Eggum (1973). The experimental diets were mixtures of the products under test with a standard protein-free diet to provide 15 g N/kg DM. The balance consisted of a 5 d preliminary and 5 d collection period; five rats (75–80 g) were used in each trial.

The true digestibility of peas was lower than that of all other varieties and TD values were generally in agreement with values reported by others (Khan *et al.* 1979). The biological value (BV) of peas and vetch peas was lower ($P<0·05$) than that of chickling vetch and field beans. The net protein utilization (NPU) was lowest for peas and vetch peas. The nutritional indexes determined in this work compare favourably with similar studies reported in the literature. It is noted, however, that no attempt was made to apply heat treatment to these products by cooking.

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FAO/WHO (1973). *Tech. Rep. Ser. Wld Hlth Org.* no. 522.
Khan, M. A., Jacobsen, I. & Eggum, B. O. (1979). *J. Sci. F Agric.* **30**, 395.

The determination of thiomolybdates in continuous cultures of rumen micro-organisms. By A. C. BRAY, N. F. SUTTLE and A. C. FIELD, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

The formation of thiomolybdates in the rumen could explain many aspects of copper, molybdenum and sulphur interactions (e.g. Suttle, 1980). It should be possible to measure quantitatively the production of mono-, di-, tri- and tetra-thiomolybdates at concentrations as low as 0·1 mg/l from their absorption spectra (peaks for MoO_3S at 288 and 392 nm, for MoO_2S_2 at 288, 322 and 394 nm, for $MoOS_3$ at 315, 398 and 465 nm and for MoS_4 at 318 and 470 nm; from Clarke & Laurie, 1980). We tried, therefore, to develop a method for estimating thiomolybdates in rumen fluid.

Continuous cultures of rumen micro-organisms were maintained using the Rusitec apparatus of Czerkawski & Breckenridge (1977). The four vessels were infused daily with 0·5 l artificial saliva containing (g/l) glucose 10·0, NH_4Cl 1·26, Na_2SO_4 0·113. The saliva also contained 0, 4, 8 or 12 mg Mo/l as Na_2MoO in vessels 1, 2, 3 and 4 respectively. A solid matrix was provided by oat hulls (20 g). Initially high background absorbance prevented the quantitative measurement of thiomolybdates but the following oxidative treatment was found to selectively remove the absorbance attributable to thiomolybdates. A sample of culture fluid (10 ml) was mixed with 0·4 ml H_2O_2 (30 g/l, w/v), allowed to stand for 20 min and centrifuged at 44 000 ***g*** for 30 min at 4°. A sample treated with water was then scanned using the oxidized sample as blank. Readings at 288 nm were negligible but peaks (absorbance maxima) were found at the longer wavelengths indicating that $MoOS_3$ and MoS_4 were the predominant species and furthermore, absorbance was approximately proportional to Mo input (see Table).

An example of absorption maximum values of supernatant fractions from Rusitec vessels using H_2O_2 treated samples as blanks

Vessel	Mo input (mg/l)	Wavelength (nm)		
		318	396	470
1	0	No absorption peaks		
2	4	0·336	0·123	0·218
3	8	0·732	0·209	0·486
4	12	1·070	0·460	0·679

Validation of the method was sought by using simultaneous equations to calculate the concentrations of $MoOS_3$ and MoS_4 present from readings at 396 and 470 nm and using the appropriate molar extinction coefficients: absorbance at 318 nm was then predicted from these concentrations and found to be in close agreement with the observed values (R 0·94, n 24). The method should be useful in establishing the importance of thiomolybdate formation in the rumen.

Clarke, N. J. & Laurie, S. H. (1980). *J. inorg. Biochem.* **12**, 37.
Czerkawski, J. W. & Breckenridge, G. (1977). *Br. J. Nutr.* **38**, 371.
Suttle, N. F. (1980). *Ann. N.Y. Acad. Sci.* **355**, 195.

The formation of tri- and tetra-thiomolybdates in continuous cultures of rumen micro-organisms and their adsorption onto 'fibre'.

By A. C. BRAY, N. F. SUTTLE and A. C. FIELD, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

When molybdate (MoO_4^{2-}) and sulphide (S^{2-}) were mixed under simulated but non-fermentative rumen conditions, tetra-thiomolybdate (MoS_4) was only formed at S^{2-}:Mo molar ratios >10 and it was concluded that the oxythiomolybdates MoS_2O_2 and $MoOS_3$ would be more likely to form in the rumen (Clarke & Laurie, 1980). The relationship between thiomolybdate production and S^{2-} and Mo concentrations has been studied in a fermentative system (Rusitec, Czerkawski & Breckenridge, 1977) under the conditions described by Bray *et al.* (1982) and using their method to detect thiomolybdates. The results in the Table indicate substantial conversion of MoO_4^{2-} to thiomolybdates, one-third of it MoS_4, over a wide range of Mo inputs and at S^{2-}:Mo values <10. Mo and S^{2-} concentrations were generally lower than those used by Clarke & Laurie (1980) and closer to those obtaining in the rumen. This suggests that non-fermentative models are inadequate for predicting thiomolybdate production in the rumen.

The thiomolybdate and sulphide concentrations of fluid sampled directly from Rusitec vessels infused with artificial saliva containing sulphate S, and various concentrations of molybdenum

Vessel	Molybdenum input (μg Mo/ml)	Thiomolybdate molybdenum μg, Mo/ml			Total: input	Sulphide μg S/ml	Sulphide: molybdenum (molar ratio)
		$MoOS_3^{2-}$	MoS_4^{2-}	Total			
2	4	2·4	1·4	3·8	0·95	16·0	12
3	8	4·4	2·4	6·8	0·85	19·2	7·2
4	12	6·4	3·0	9·4	0·78	14·6	3·6

Limitations of the Rusitec model were indicated by the fact that Mo was found predominantly in the liquid phase whereas in vivo it is predominantly in the solid phase (Grace & Suttle, 1979). The Rusitec model had a low solid:liquid phase value and when the solid phase was increased, more Mo was bound to it. When the oat hull matrix from vessels 1 to 4 was removed after 24 d it was found to contain 3, 53, 197 and 195 mg Mo/kg DM, respectively, i.e. only 1·6 to 3·2% of the added Mo. When fresh hulls were introduced, they were found to have adsorbed 11–13% of the added Mo after 2 d. When washed ground oat hulls were shaken for 2–4 h with effluent from vessel 4 (40 to 133 g/l), at least 50% of the thiomolybdate Mo was apparently absorbed. Ground hay also absorbed thiomolybdates but pure cellulose did not. The effect of thiomolybdate distribution on Cu and Mo absorption merits further study.

Bray, A. C., Suttle, N. F. & Field, A. C. (1982). *Proc. Nutr. Soc.* **41**, 66A.
Clarke, N. J. & Laurie, S. H. (1980). *J. inorg. Biochem.* **12**, 37.
Czerkawski, J. W. & Breckenridge, G. (1977). *Br. J. Nutr.* **38**, 371.
Grace, N. D. & Suttle, N. F. (1979). *Br. J. Nutr.* **41**, 125.

A study in vitro of the effect of picolinic acid on metal translocation across model membranes. By P. J. AGGETT, P. K. FENWICK and HELEN KIRK, *Department of Physiology, University of Aberdeen.*

It has been proposed that picolinic acid (PA; pyridine-2-carboxylic acid), a tryptophan metabolite, has a physiological role in the intestinal absorption of zinc and that it is of benefit in the treatment of acrodermatitis enteropathica (Evans, 1980, Krieger & Evans, 1980).

In this investigation a possible mechanism and its specificity for these reported effects was sought by studying the ability of PA to transfer Zn and other divalent cations across pure lipid membranes using liposomes as model membranes. The efflux of entrapped metals from these vessels was monitored using either radiolabels (^{42}Ca, ^{65}Zn) or measuring the formation of extraliposomal colorimetric complexes (Aggett *et al.* 1982).

Extraliposomal PA increased the efflux of Zn, Cu, Co, Mn, Ni, Cd, Pb, Fe(II) and Ca, but this efflux was reduced by intraliposomal PA and by the presence of equimolar metal solutions in the extraliposomal medium. In a two-phase extraction study PA failed to increase the metal content of the organic phase but increased the solubility in aqueous solvent at alkaline pH.

It is concluded that PA does not have all the properties of an ionophore and that if it does enhance intestinal absorption of metals, this ability depends on an as yet undefined mechanism. Our results are consistent with the failure to confirm any enhancement of intestinal Zn absorption by PA in rats (Jackson *et al.* 1981), man (Casey *et al.* 1980) or cattle with Adema disease (Flagstad, 1981).

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

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Casey, C. E., Hambidge, K. M. & Walravens, P. A. (1980). In *Trace Substances in Environmental Health*, XIV, p. 80 [D. D. Hemphill, editor]. University of Missouri.
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Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1981). *Br. J. Nutr.* **46**, 15.
Krieger, I. & Evans, G. W. (1980). *J. Pediat.* **96**, 32.

Perinatal mortality in zinc deficient rats is associated with significantly reduced utero–placental blood flow. By S. C. CUNNANE, *Nuffield Laboratories of Comparative Medicine, Institute of Zoology, Regent's Park* and E. MAJID and J. SENIOR, *School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP* and C. F. MILLS, *Department of Nutritional Biochemistry, Rowett Institute, Aberdeen AB2 9SB*

The mechanism by which zinc deficiency during pregnancy causes high perinatal mortality in rats is unknown. However, it has recently been shown that there is a 50–150% increase in the conversion of [^{14}C]arachidonic acid to '2 series' prostaglandins (PGs) in the placenta of Zn-deficient parturient rats (Cunnane, 1981). Since PGF_{2alpha} and PGE_2 are utero–placental vasoconstrictors (Clark *et al.* 1981), it is possible that excess synthesis of '2 series' PGs may contribute to reduced utero–placental blood flow in Zn-deficient rats increasing perinatal mortality at term.

Utero–placental blood flow was measured using labelled microspheres (Phaily & Senior, 1978). On day 22 of gestation, five control rats fed a Zn adequate diet (40 μg Zn/kg throughout gestation) and six Zn-deficient rats (10 μg Zn/kg for the first 2 weeks of gestation followed by 0·5 μg Zn/kg for the remainder of gestation) were anesthetized with nembutal. The left ventricle of the heart was cannulated via the left carotid artery for microsphere injection and the left femoral artery was cannulated for blood withdrawal. Approximately 100 000 ^{46}scandium labelled microspheres (15 μm diameter, New England Nuclear) were injected into the arterial circulation via the carotid cannula. Blood was withdrawn via the femoral cannula for about 1 min at 0·5 ml/min. The rats were then killed and placentas, uteri, adrenals, kidneys and a piece of lung were removed, weighed and their radioactivity measured in a gamma-counter.

Total cardiac output was decreased by 50% in the Zn-deficient rats (45 *v* 88 ml/min, respectively). The distribution of cardiac output was significantly decreased to the placenta, uterus and adrenals (33, 52 and 48% of control, respectively) in the Zn-deficient rats, but was increased to the kidneys (166% of control). Blood flow (ml/min and ml/min per g body-weight) was also significantly decreased to the placenta, uterus and adrenals of the Zn-deficient rats (16, 30 and 21% of control, respectively), but flow to the kidneys and lung remained unchanged from control values.

These observations demonstrate that in Zn-deficient rats at term, the cardiac output and blood flow to the utero–placental vascular bed is significantly decreased. Blood flow to the kidneys and lungs was not decreased in Zn deficiency. This suggests that the effect of Zn deficiency on circulatory function is not systemic, but rather is specifically related to the uterus and placenta, and related endocrine organs such as the adrenals which may also have an important function in the termination of pregnancy. Perinatal mortality in Zn deficiency would therefore seem to be dependent on two possibly related events; (1) altered metabolism of arachidonic acid to vasoconstrictor PGs and (2) decreased utero–placental blood flow.

S.C.C. was a fellow of the Lalor Foundation, Wilmington, Delaware, USA.

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Influence of anions and organic ligands on the intestinal absorption of zinc in vitro. By C. J. SEAL and F. W. HEATON, *Department of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ*

Because methods for measuring mineral absorption in vivo are time-consuming and costly, we used a simple intestinal sac technique to study the effect of variation in chemical state on zinc uptake.

Everted sacs from the duodenum and ileum of adult male Wistar rats were filled with Zn-free 0·15 M-Tris/Krebs buffer solution (pH 7·3) and incubated in the same buffer solution containing different salts at a constant concentration of 3×10^{-4} M-Zn. Organic ligands were added at a concentration of $1\cdot5\times10^{-2}$ M. After incubating for 30 min at 37°, the Zn present in the sac contents was determined by atomic absorption flame-photometry.

Chemical state of Zn	No. of observations	Zn uptake (μg/g dry weight tissue in 30 min)			
		Duodenum		Ileum	
		Mean	SEM	Mean	SEM
$ZnCl_2$	50	23·4	0·9	13·0	0·5
$ZnSO_4$	16	34·5	2·1***	15·4	1·0*
$Zn_3(PO_4)_2$	8	14·7	1·3***	13·2	0·9
Zn acetate	8	35·5	4·7***	14·2	1·0
Zn citrate	6	11·9	1·0***	9·6	0·2**
$ZnCl_2$ + aspartic acid	8	27·4	1·8*	17·1	1·3**
$ZnCl_2$ + cysteine	8	19·3	2·3	13·5	1·0
$ZnCl_2$ + glutamic acid	8	22·3	1·9	13·4	1·2
$ZnCl_2$ + histidine	8	35·7	2·1***	17·7	2·9**
$ZnCl_2$ + tryptophan	8	20·3	1·6	10·7	0·7
$ZnCl_2$ + galactose	8	15·5	1·7***	12·3	0·6
$ZnCl_2$ + lactose	8	15·8	0·8***	12·8	1·2
$ZnCl_2$ + 2-picolinic acid	5	94·8	12·2***	33·3	2·2***
$ZnCl_2$ + 4-picolinic acid	4	11·1	1·6***	8·2	0·7**

Value significantly different from $ZnCl_2$; *$P<0·05$, **$P<0·01$, ***$P<0·001$.

Taking Zn chloride as the standard of reference, sulphate and acetate anions increased Zn uptake, but phosphate and citrate reduced it, with the effects being more marked in duodenum than ileum. Among the organic components of foodstuffs studied, histidine and aspartic acid increased Zn absorption, whereas galactose and lactose tended to reduce it. The effect of picolinic acid is interesting because the 2-isomer greatly stimulated Zn uptake but the 4-isomer inhibited it.

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Zinc absorption in gluten-sensitive enteropathies. By R. W. CROFTON[1], S. C. GLOVER[1], P. J. AGGETT[2], N. A. G. MOWAT[1], S. W. B. EWEN[3] and C. F. MILLS[4], [1]*Department of Medicine,* [2]*Physiology and* [3]*Pathology, University of Aberdeen* and [4]*Department of Nutritional Biochemistry, Rowett Research Institute, Bucksburn, Aberdeen*

Low plasma zinc concentrations have been described in coeliac disease and it has been suggested that these reflect low body Zn status in this condition. In this study Zn absorption in gluten-sensitive enteropathies (coeliac disease and dermatitis herpetiformis (DH)) has been investigated by the 'Zn tolerance test'. The value of this test in assessing intestinal function was determined by comparing it with other tests of absorption and by correlating it prospectively with the response of the small intestinal mucosa to the withdrawal of gluten from the diet.

Twenty-two patients with coeliac disease and eleven patients with DH were studied. Jejunal biopsies were obtained by Crosby capsule and subjected to morphometric analysis. Zn absorption was assessed by taking blood samples over 6 h following the ingestion of 50 mg elemental Zn as the sulphate. The plasma Zn increments were plotted against time and the areas under the curves at 3 h (AUC_3) and 6 h (AUC_6) calculated.

The results of the AUC_3 are summarized in the Table. The AUC_6 showed a similar trend. Following the withdrawal of gluten from the diet whilst under close dietary supervision the AUC_3 and AUC_6 improved significantly.

Zn absorption in coeliac disease and dermatitis herpetiformis (AUC_3; μmol/l per h)

	At entry			One year on diet			Five years on diet		
	n	Mean	SEM	*n*	Mean	SEM	*n*	Mean	SEM
Healthy volunteers	15	401·1	12·4						
Untreated coeliac disease	12	186·5	22·0**	14	365·0	58·5^{NS}	4	405·6	61·4^{NS}
'Treated' coeliac disease	10	396·2	64·4^{NS}						
Dermatitis herpetiformis	11	205·8	26·2*	4	308·2	41·3*	4	481·7	41·6^{NS}

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

Whereas conventional tests of absorption (e.g. D-xylose absorption, faecal fat excretion) did not correlate with the villus-crypt ratio (VCR) the AUC_3 (r 0·42, $P<0.05$) and AUC_6 (r 0·43, $P<0.05$) did do so.

This investigation confirms that zinc absorption is impaired in untreated coeliac disease and DH and furthermore it appears to be a more sensitive index of malabsorption than conventional tests.

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

The effects of food deprivation and bile salt depletion on bile flow rate, the secretion of bile salts and the biliary excretion of manganese, copper, iron and zinc in the bile of steers. By H. W. SYMONDS and C. B. MALLINSON, *ARC Institute for Research on Animal Diseases, Compton, Newbury, Berkshire RG16 0NN*

Four adult steers weighing 640–700 kg were deprived of food but not water for 5 d. Bile flow and concentrations of manganese, copper, iron and zinc in bile were measured during consecutive 30 min periods for 7 h each day during 2 (control) d before food deprivation and during the 3rd, 4th and 5th d of food deprivation and during the 1st d of refeeding. The bile flow (ml/min) and mean excretion rates (μmol/min±SE) of Mn, Cu, Fe and Zn during the control and experimental periods are shown in the Table. The principal changes during food deprivation were an approximately 40% reduction in bile flow rate, an 80% decrease in the excretion of Mn, a 40% decrease in the rate of excretion of Zn, and a 60% increase in the rate of excretion of Cu.

			Mn (μmol/min)		Cu (μmol/min)		Fe (μmol/min)		Zn (μmol/min)	
Day	Bile flow (ml/min)	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	9·3	0·5	0·264	0·039	0·021	0·003	0·067	0·008	0·059	0·006
			(% of control values)							
3	76·6	5·4	47·0	7·6	73·2	12·7	75·2	7·1	97·4	6·8
4	70·8	8·5	28·0	3·4	102·4	22·4	99·2	7·5	56·4	8·0
5	62·1	8·5	16·3	3·4	136·6	19·0	79·7	6·9	54·7	12·6
Refed	98·6	8·6	87·9	10·9	151·2	29·3	91·7	4·8	92·3	8·7

The enterohepatic circulation (EHC) of bile salts was interrupted for up to 2 d in seven steers. Within 150 min bile flow decreased by approximately 78% and the secretion rate of total-cholate (Irvin *et al.* 1944) decreased from approximately 1000 to 20–30 μmol/min. The amount of total cholate within the enterohepatic circulation system was estimated to be 194±6 μmol/kg body-weight. When the EHC was re-established the bile salt secretion rate increased exponentially during the first 6 h so that the rate doubled within approximately 200 min. The effect of bile salt depletion on the excretion rate of Cu, Zn and Fe was variable; in some animals there was no change and in others a reduction. The excretion of Cu decreased by approximately 50%. The excretion of Mn decreased consistently by between 75 and 95% and it increased to equal or exceeded the control rate when the EHC was re-established.

These results suggest that the excretion of Mn in bile is related directly to the absorption of Mn from the gastrointestinal tract and to the secretion of either bile salts or some other component of bile.

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Studies on the palatability of salt and urea in sheep. By W. L. GROVUM and H. W. CHAPMAN (Introduced by H. S. BAYLEY), *Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W2*

The two-choice preference test has been widely used in studies of palatability in ruminants (Bell, 1959; Goatcher & Church, 1970) but the results are confounded since the animals drink the test solution and it is not known whether an animal's reaction to a chemical is due to oral–pharyngeal sensations or to post-ingestive factors.

Six mature Suffolk wethers with oesophageal fistulas closed by plugs (Chapman, 1964) were given a basal diet of medium quality mixed hay supplemented with salt in blocks. On each day of an experiment, the sheep were fed hay from 8–9.00 h, deprived of food for 5·5 h and then, with oesophageal plugs removed, given free access to test diets for 30 min. These diets were ground and pelleted lucerne (LP) (Expt 1) and LP containing (a) 10% NaCl (Expt 2), (b) 0, 5, 10, 15 and 20% NaCl (Expt 3), (c) 1% urea (Expt 4) and (d) 0, 1, 2, 4 and 8% urea (Expt 5). In Expts 1 and 2, intakes of the test diets increased for 6 and 8 d respectively and then fluctuated about a plateau. In Expt 3, which was done according to a 5×5 Latin Square design, the sheep may have had a slight preference for NaCl even at 20% of the diet since they ate 1136, 1433, 1309, 1496 and 1485 g/30 min of the 0, 5, 10, 15 and 20% salt pellets respectively ($P>0·05$). Goatcher & Church (1970) reported that a 2·2% solution of NaCl was strongly rejected by sheep in a two-choice preference test. The sheep ate similar quantities of the 1% urea pellets on six successive experimental days (Expt 4), but in Expt 5 which was another 5×5 Latin Square, they discriminated equally against all levels of urea since their mean intakes were 1942, 1424, 1510, 1360 and 1387 g/30 min for the 0, 1, 2, 4 and 8% urea pellets respectively, where the control value was significantly different from all the other values ($P<0·05$). These results clearly demonstrate that gustatory and post-ingestive factors can and must be separated in future studies of palatability.

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The effect of haemonchosis and blood loss into the abomasum on N digestion in sheep. By J. B. ROWE, *ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG* and E. M. ABBOTT, J. D. DARGIE and P. H. HOLMES, *Department of Physiology, Veterinary School, University of Glasgow, Glasgow*

Haemonchus contortus is an abomasal parasite which is important throughout the world. The impact of blood loss into the abomasum on the digestion of N in sheep infected with *Haemonchus contortus* was investigated in the following manner. Nine adult sheep were prepared with cannulas in the rumen, duodenum and ileum and divided into three groups—a control group; an infected group (1000 *Haemonchus contortus* larvae/kg body-weight) 4 weeks before the experiment; and a third group (simulated infection). In the latter group, blood was removed from the jugular vein (200 ml/d) and slowly transfused into the abomasum via an indwelling catheter over the subsequent 24 h. This procedure was started 10 d before the study and continued throughout the experiment. A diet consisting of equal amounts of chopped hay and pellets: (g/kg) ground straw 800; soya-bean meal 100; molasses 60; minerals + vitamins 40) was fed *ad lib.* to the parasitized sheep and the intakes of the control and 'bled' groups were matched to those of the parasitized animals. N balance was measured in all animals over an 8 d collection period. The flow of N from the forestomachs and an ileal digesta was measured relative to the inert markers ^{103}Ru-P and ^{51}Cr-EDTA (Faichney, 1975).

There were considerable differences in the level of infection in the three parasitized sheep (100–7000 eggs/g faeces) and feed intakes were depressed in the two animals with the heavier worm burdens (967, 665 and 147 g DM/d). In animals with the simulated infection there was an increase of approximately 6 g N/d in the digesta flowing from the abomasum, in comparison to the control group. There was a similar increase in the parasitized sheep. However, there were no marked differences in ileal N flow, or faecal or urinary N excretion as a result of either treatment. There was a significantly ($P<0.01$) higher rate of cell turnover in the abomasal wall of the parasitized animals measured using ^{3}H-thymidine incorporation.

The results suggest that blood protein lost into the abomasum may be efficiently reabsorbed from the small intestine, even in heavy infections. This indicates that the depressed productivity associated with the haemonchosis may result primarily through reduced feed intake and increased energy utilization in tissue and blood regeneration rather than through a protein drain on the animal from blood loss into the abomasum.

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The excretion of allantoin by ruminants in relation to protein entering the abomasum. By S. SIBANDA and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD* and E. STORM and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The urinary excretion of purine derivatives, especially allantoin, has been suggested as a useful indicator of net synthesis of protein by rumen microbes (Topps & Elliott, 1965). Variation in the endogenous excretion, however, may limit the use of allantoin as an indicator of microbial synthesis (Smith, 1975). Urine samples from several experiments were analysed to examine the effects of energy and protein nutrition on the endogenous excretion of purine derivatives and the closeness of the relationship between nucleic acid infused into the abomasum and urinary allantoin.

Samples were obtained from the following animals: two dry cows infused ruminally with four levels (i.e. 0·5, 1·0, 1·5 and 2·0×maintenance) of volatile fatty acids (VFA), each given with a maintenance level of casein, infused abomasally; the same cows, whilst lactating, given three mixtures of VFA, each with casein, all infused at twice maintenance level; two steers given maintenance level of VFA with or without casein at the maintenance level; three sheep infused ruminally with one level of VFA (1·25×maintenance) and abomasally with four levels of rumen microbial preparation and two sheep given the same VFA with and without an egg preparation.

Excretion of endogenous allantoin N by the cows, which ranged from 30 to 47 mg/kg$^{0·75}$ per d, was influenced by the level of energy and protein infused. The lowest values were obtained when VFA and casein were given at twice the maintenance level while the highest values resulted when no nutrients were infused. Excretion of endogenous allantoin N by the steers was considerably less than that of the cows, the values ranging from 21 to 25 mg/kg$^{0·75}$ per d.

With sheep, the excretion of allantoin N (AN) was directly related to the nucleic acid N (NAN) infused, the regression being AN (mg/kg$^{0·75}$ per d) = 0·009 NAN (mg/d) + 9·38 (r 0·945, RSD 2·46, $P<0·001$). The mean excretion of endogenous allantoin N by the sheep was 9 mg/kg$^{0·75}$ per d and if this is taken into account the corresponding relationship for exogenous allantoin N (EAN) is EAN = 0·010 NAN + 1·36 (r 0·895, RSD 2·96, $P<0·001$). It is concluded that allantoin excretion may be a good indicator of microbial synthesis provided account is taken of differences in endogenous excretion between species and in animal production and plane of nutrition.

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The flow of N from the rumen of cows and steers maintained by intraruminal infusion of volatile fatty acids. By E. R. ØRSKOV and N. A. MACLEOD, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The technique of intragastric infusion (Ørskov *et al.* 1979) has enabled us to measure basal N excretion of ruminants uncomplicated by the activity of micro-organisms in the forestomach (Ørskov & MacLeod, 1982). The technique has now been used to estimate the flow of non-ammonia N from the rumen when there is no fermentation in that organ. Information on this aspect is required to ascertain the extent to which N addition between the mouth and the duodenum arises from sloughing of rumen epithelial tissue. Dinsdale *et al.* (1980) have shown that there were no differences in the mitotic index of rumen epithelial tissue in normally fed animals and those maintained on infusion. Some of the protein could also originate from epithelial abrasion from the respiratory tract, mouth and oesophagus.

The rumen volumes and liquid outflow rates were determined using polyethylene glycol with two cows at two stages of pregnancy (130 and 240 d) and with two steers at different stages of maturity.

The outflow of N from the rumen of cows and steers given N-free nutrients by intragastric infusion

Animals	Live weight (kg)	Rumen volume (l)	N content (g/kg)	N flow (g/d)	Non NH_3-N (g/d)	Non NH_3N (mg N/kg $W^{0.75}$ per d)
Dairy cows (2)	654	48·6	0·153	9·8	6·9	53
Dairy cows (2)	693	62·1	0·194	14·5	10·7	79
Steers (2)	347	36·4	0·150	7·0	5·2	65
Steers (2)	387	50·8	0·187	7·1	5·0	57
Steer (1)	380	53·1	0·083	6·6	5·1	59

In the Table results are given from periods in which the animals were sustained by the infusion of volatile fatty acids in amounts sufficient to provide the estimated energy requirement for maintenance and no protein was given. The outflow of non ammonia-N expressed in relation to metabolic body-weight varied from 57 to 79 mg/kg $W^{0.75}$ per d. Microscopic examination of the protein indicated that it consisted mainly of abraded epithelial cells and showed that a substantial proportion of N added between the mouth and the duodenum is contributed in this way. The extent to which such material is degraded normally in the rumen or digested in the small intestine and utilized as a source of amino acids is not known.

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Comparison of rumen and faecal sampling procedures for calculating the retention time of digesta markers in the rumen of steers. By J. J. F. MIRA, *North of Scotland College of Agriculture, Aberdeen AB9 1UD* and J. C. MACRAE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Compartmental analysis of marker excretion in the faeces is now widely used to obtain estimates of the retention time of digesta in various segments of the gastro-intestinal tract of ruminants (see Review by Warner, 1981). Indeed Grovum & Williams (1977) have demonstrated that when a pulse dose of liquid phase marker ^{51}CrEDTA and a particulate marker ^{144}Ce–^{144}Pr were introduced into the rumen of sheep the rate of appearance of each marker in the faeces could be equated to their respective rates of disappearance from the rumen. Lemerle *et al.* (1980) reported similar observations when using CrEDTA and Ru-phenanthroline in cattle given poor quality pasture hay.

In a recent study with three one-year-old steers fed at 12 hourly intervals on rations of long or chopped (medium and fine) straw plus rolled barley (2 kg/d), ^{103}Ru-P (25 μCi) and ^{51}CrEDTA (120 μCi) were both introduced into the rumen as a pulse dose 2 h after a morning feed. Samples of rumen digesta were withdrawn by suction from different parts of the rumen 1 h before and 1 h after each feed for the next 48 h and grab samples of faeces were also obtained every 6 h over the first 60 h and 12 hourly thereafter for a further 3 d. The Table gives the retention times (h) of the two markers for each dietary treatment calculated either from the rate of fall of marker concentration in the rumen or from the rate of appearance of marker in the faeces.

	Long straw	Chopped straw		SED
		Medium	Fine	
Straw DM intake (kg/d)	2·63	2·69	2·65	0·392
Digestibility coefficient of ration	0·64	0·64	0·61	0·015
Retention time in rumen (h):				
Particulate matter (faeces)	28·8	27·9	27·6	3·63
(rumen)	24·0	25·0	22·9	2·75
Liquid phase (faeces)	11·9	11·8	11·5	1·05
(rumen)	12·5	10·5	11·4	1·27

Processing of the straw had little effect on the retention time of either marker in the rumen. As was expected the retention time of the ^{51}CrEDTA was much shorter ($P<0·001$) than that of the ^{103}Ru-P on all diets. However, whilst the liquid phase retention times calculated from the rumen analyses were similar to those calculated from the faecal analyses, this was not so with the particulate phase. The ^{103}Ru-P retention times calculated from the changes in ^{103}Ru-P per g DM rumen digesta were significantly shorter ($P<0·05$) than those calculated from changes in ^{103}Ru-P per g DM faeces. These differences may indicate that in cattle given straw plus cereal diets the particulate phase of rumen digesta is not contained in a single, easily sampled, compartment (see Ellis *et al.* 1979).

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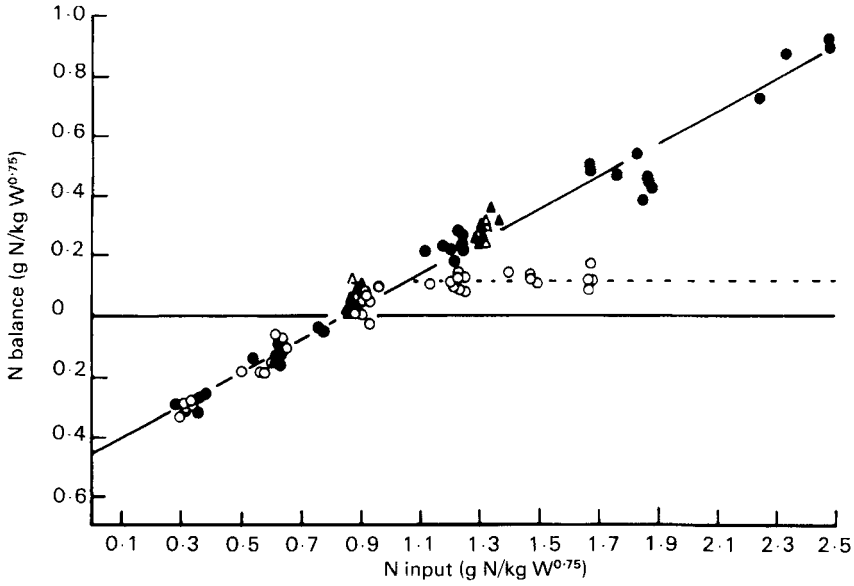
Biological value and digestibility of rumen microbial protein in lamb small intestine. By E. STORM and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Most work on utilization of rumen microbial protein has previously been carried out with rats due to the difficulty of obtaining quantities large enough for estimation of nutritive value in ruminants. However, a method for large-scale isolation of rumen micro-organisms (RMO) has been developed (Storm & Ørskov, 1979).

Ten lambs were sustained by the intragastric infusion of volatile fatty acids (Ørskov *et al.* 1979), at one of three levels of energy (see Fig.) and were infused with one of six different amounts of isolated RMO. The relationship between N balance and N input shows that at the level of 430 kJ/kg $W^{0.75}$ energy was limiting N retention at inputs higher than 1 g N/kg $W^{0.75}$. Excluding these values there was a highly significant ($P<0.001$) linear relationship between N retention (y) and N input (x), which could be described by the expression $y=0.543\ x-0.457$ (RSD 0.037), where the values are expressed in g N/kg $W^{0.75}$. The SE of the regression coefficient was 0.008.



The relationship between N input in RMO and N balance in eighteen lambs. The gross energy infused was 430 kJ (○) Expt 1, 860 kJ (●) Expt 2, and 750 kJ (△▲) Expt 3 and 3b. All values expressed on kJ/kg $W^{0.75}$.

In a subsequent experiment three lambs were infused with none or one of four different levels of RMO. The lambs were fitted with ileal cannulas and the disappearance of amino-acid N (AA-N) determined. The true digestibility of microbial AA-N in the small intestine was 0.847 ± 0.012.

If the assumption is made that the absorbed nucleic acids and other non-protein N in RMO was not utilized for N retention (Bird, 1972), it is possible to show that the efficiency of utilization of absorbed AA-N from RMO for the lambs used was 0.801.

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The establishment of equilibrium conditions in the digestive tract of the sheep after dietary change. By R. F. E. AXFORD, R. A. EVANS, K. GHEBREMESKEL, N. SIULAPWA and A. VERA, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

It is customary to allow a period of equilibration to pass after a dietary change has been imposed before collecting samples in a balance study using sheep. There is no general agreement as to the time delay needed before the collected samples can be held to be representative of the newly established state, although the majority of workers choose a preliminary period of between 7 and 14 d (Charlet-Lery, 1963).

We have been studying the passage of materials through the digestive tract of sheep fitted with re-entrant cannulas in the proximal duodenum and fed at 3 hourly intervals. Representative samples of duodenal digesta were collected continuously by an automated sampling device, and faeces were also collected daily. During sampling periods of up to 70 d, the animals were subjected to abrupt changes in diet, involving either quantity or quality of intake. Sampling continued throughout the changeover periods. Each diet was fed for 10 or 20 d before the next change. The passage of dry matter, organic matter, soluble carbohydrates, pectin, hemicellulose, cellulose, total nitrogen, non-ammonia nitrogen, and gross energy in the duodenal flow and of dry matter, organic matter and gross energy in the faeces were studied by cumulative sum analysis and it was found that in almost all cases the new equilibrium situation was established by the fourth day after the dietary change. It was considered that the mean flows of the various components for the last 3 d on each diet would be acceptable as representative by other workers since they were preceeded by equilibrium periods of 7 to 17 d. These means were compared by analysis of variance and paired *t* tests with the mean flows for days 4, 5 and 6 after the dietary change, and were found to be indistinguishable. This result was obtained whether the dietary change induced an increase or decrease in dietary flow of the component studied.

The results described indicate that it might be possible to reduce the duration of a balance trial. This would have the advantage that several diets could be compared in the same animal within a short period, while food, physiological state and environmental conditions are sensibly constant.

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Possible effect of molybdenum on fertility in the cow. By M. PHILLIPPO, W. R. HUMPHRIES, I. BREMNER, B. W. YOUNG, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

There has been controversy about the possible effect of low copper status on fertility in the cow and whether Cu therapy has any beneficial effect upon fertility. Previous results had shown that Cu treatment in herds of low Cu status did not affect conception (Phillippo *et al.* 1982). The following observations suggest that reproductive performance may indeed be impaired in Cu-deficient cattle provided that their Cu status is reduced by high molybdenum intakes.

Sixteen Hereford-Friesian heifers were randomly allocated at 100–180 kg live weight to four groups and given a barley-straw ration for 32 weeks. The diet contained 4 mg Cu, 2·8 g sulphur and 250 mg iron/kg DM and was supplemented with 0 or 800 mg Fe (as saccharated ferrous carbonate), and 0 or 5 mg Mo (as ammonium molybdate)/kg (see Table). One animal died of bloat after this period and oestrus in the remaining animals was synchronized with a synthetic prostaglandin. They were artificially inseminated and then run with a bull for 16 weeks. Pregnancy was diagnosed by rectal palpation and by plasma progesterone analyses 18 weeks after insemination.

All the Fe and Mo treatments significantly reduced the Cu concentrations in both liver and plasma to very low levels at 32 weeks but there were no differences between the Fe, Mo or Fe + Mo groups (see Table). Mo supplementation significantly reduced live-weight gain. The number of animals that conceived was significantly lower in the Mo supplemented groups (two out of eight) compared to the two non-Mo supplemented groups (seven out of seven, $\chi^2 = 5{\cdot}65$ $P < 0{\cdot}05$). Iron treatment by itself did not have any effect even though the Cu status was as low as the Mo-groups.

Supplement . . .	Control		Fe		Mo		Fe + Mo	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver Cu (mg/kg DM)	66·9	19·5	3·7	0·5	3·1	0·9	2·2	0·2
Plasma Cu (μg/ml)	1·03	0·04	0·23	0·04	0·16	0·02	0·16	0·04
Live weight (kg)	358	31	380	18	327	13	308	15
No. pregnant (at 18 weeks)	4/4		3/3		0/4		2/4	

The number of animals used in this experiment is small and any conclusion must be tentative. They do indicate that Mo might cause poor reproduction in the cow. Low Cu status *per se* does not seem to inhibit reproduction since the 'Fe-alone' group had Cu concentrations as low as those in the Mo and Fe–Mo animals. Further work is needed to confirm these observations and to determine whether the apparent Mo effects are direct, as suggested by Thomas & Moss (1951) in the bull, are indirect due to the inhibition of growth, or reflect more severe Cu deficiency at specific functional sites.

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The clearance of copper from the plasma of cattle and its excretion in bile during the intravenous infusion of copper sulphate solutions. By LYNNE L. CHARMLEY, H. W. SYMONDS and C. B. MALLINSON, *ARC Institute for Research on Animal Diseases, Compton, Newbury, Berkshire RG16 0NN*

Copper sulphate solutions were infused into a jugular vein of two Friesian steers (A and B) which had been surgically prepared to enable bile to be collected and its flow rate measured (Symonds *et al.* 1982) and also into a jugular and a mesenteric vein of one Friesian cow with cannulas in mesenteric, portal, hepatic, jugular and carotid blood vessels. Steer A (body-weight 495 kg) received 20·3 μmol Cu/min for 6 h, and steer B (body-weight 300 kg) 20·1 μmol Cu/min for 5 h. The initial plasma Cu concentrations were 14·4 and 17·3 μmol/l in steers A and B respectively. Systemic plasma Cu concentrations increased only during the first 30 min of the infusions and then remained constant. The plateau values were (+ SEM) 36·5±0·2 and 34·8+0·2 μmol Cu/l for steers A and B respectively. After the infusions the plasma Cu levels decreased rapidly ($t_{\frac{1}{2}}$=1·4±0·3 min) to the pre-infusion concentrations.

During the infusions into steers A and B the excretion rates of Cu in bile were 0·043±0·005 and 0·058±0·013 μmol/min respectively, which were not significantly greater than the normal excretion rates. During the 10 d after the infusion, the increase in biliary Cu excretion in steer A accounted for less than 4% of the Cu infused. After the infusion steer B suffered cholestasis, dehydration and loss of appetite and no measurements were made. In both steers, during the infusions, the rate of excretion of Cu in urine was low (less than 0·1 μmol/min).

The cow (body-weight 460 kg) was given one 4 h infusion of 20·9 μmol Cu/min into a jugular vein and another, identical, infusion into a mesenteric vein 2 d later. During both infusions the arterial plasma Cu concentration increased rapidly to an equilibrium value within 30 min; but the increase (14·5±0·5 μmol/l) was greater during the intrajugular infusion than during the intramesenteric infusion (7·7± 0·2 μmol/l). During the intramesenteric infusion the liver must have removed approximately 46% ($\frac{14\cdot5-7\cdot7}{14\cdot5}\times 100$) of the Cu infused. During the equilibrium state of the intrajugular infusion the increases in the concentrations of Cu in the portal and hepatic venous plasmas were 12·1±0·3 and 10·5±0·2 μmol/l respectively, suggesting that Cu was being removed by the gastrointestinal tract as well as by the liver.

These observations show that approximately 95% of infused Cu is cleared rapidly from bovine systemic plasma. However, small quantities of this Cu are excreted in urine and bile during the 10 d after the infusions.

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The effects of iron and sulphide on copper metabolism in rats. By I. BREMNER, B. W. YOUNG and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Increased dietary iron intakes have been found to induce severe copper deficiency in cattle but not in rats, suggesting that an important site of interaction between Fe and Cu could be in the rumen (Humphries *et al.* 1981; Bremner & Young, 1981). Since the Cu–molybdenum interaction in cattle also occurs principally in the rumen and is markedly influenced by ruminant sulphide production, it seemed possible the S^{2-} could also modify the Cu–Fe interaction. A study has therefore been made of the effects of dietary supplementation with Fe or S^{2-} or both on Cu metabolism in rats.

Four groups each of six male rats were given for 2 weeks a semi-synthetic diet with 3 mg Cu/kg and supplemented with 50 or 500 mg Fe (as $FeSO_4.7H_2O$) and 0 or 288 mg S^{2-} (as CaS)/kg. The rats were then given a single oral dose of ^{64}Cu, killed 4 h thereafter, and the ^{64}Cu content of the gut-free carcass was measured (see Table). Dietary supplementation with Fe alone had no effect on ^{64}Cu absorption but Fe did exacerbate the inhibitory effect of S^{2-} on ^{64}Cu absorption. The distribution of absorbed ^{64}Cu between liver, kidneys, blood and the remainder of the carcass was not influenced by the dietary treatments.

Dietary Fe (mg/kg)	50	50	500	500
Dietary S^{2-} (mg/kg)	0	288/144	0	288/144
Expt 1				
^{64}Cu absorption (% dose)	32·7 ±4·6	11·5 ±0·8	36·8 ±3·6	3·3 ±0·8
Expt 2				
Liver Cu (mg/kg)	3·3 ±0·1	3·6 ±0·1	4·3 ±0·3	2·1 ±0·4
Kidney Cu (mg/kg)	11·2 ±1·2	6·3 ±0·8	9·8 ±0·5	3·6 ±0·2
Plasma Cu (mg/l)	1·08±0·03	0·86±0·01	0·93±0·02	0·19±0·05

In a second experiment, four groups each of five male rats were given for 7 weeks diets with 50 or 500 mg Fe (as $FeSO_4.7H_2O$) and 0 or 144 mg S^{2-} (as CaS)/kg. Supplementation with Fe or S^{2-} alone had no major effects on plasma or tissue Cu concentrations but simultaneous addition of Fe and S^{2-} to the diet reduced Cu concentrations in plasma, liver and kidneys by 40–80% (see Table).

It appears therefore that there may indeed be some combined effects of Fe and S^{2-} which can restrict the availability of Cu to rats, although the mechanism whereby this occurs is not yet clear. The possibility that dietary sources of S may influence the antagonistic effects of Fe upon Cu utilization by cattle is being investigated.

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The importance of soil type and dietary sulphur in the impairment of copper absorption in sheep which ingest soil. By N. F. SUTTLE, *Moredun Research Institute, Gilmerton Road, Edinburgh EH17 7JH* and P. W. ABRAHAMS and I. THORNTON, *Applied Geochemistry Research Group, Imperial College, London SW7 2BP*

Ingested soil lowers the absorption of copper by sheep (Suttle *et al.* 1975) but the mechanism and hence possible influences of soil type and dietary composition are unknown. The effects of three contrasting soils, a 'clay', a 'chalk' and a 'sand' were therefore compared using basal diets high or low in sulphur (4 and 1 g S/kg DM in Expts 1 and 2, respectively) in similar repletion experiments to that used initially (Suttle *et al.* 1975). The three soils varied in their iron content and since soil Fe might be an antagonistic factor, an Fe treatment was included in each experiment. Four groups of four or five hypocupraemic ewes were given diets containing 100 g/kg DM of the chalk, clay and sand or $FeSO_4$: the supplements provided 2400, 1440, 140 and 800 mg Fe/kg diet DM, respectively. A fifth group received no supplement to the basal diet which contained 9·9 and 6·1 mg Cu/kg DM in Expts 1 and 2, respectively. The responses in plasma Cu (mg/l) after 21 d and derived absorption coefficients for dietary Cu are given in the Table.

(Derived Cu absorption coefficients are given in parentheses)

Supplement . . .	None	Chalk	Clay	Sand	$FeSO_4$	SE
Expt 1	0·21 (0·031)	0·03 (0·017)	0·02 (0·017)	0·19 (0·030)	−0·09 (0·015)	0·03
Expt 2	0·46 (0·077)	0·34 (0·064)	0·44 (0·075)	— —	0·127 (0·035)	0·06

In Expt 1 the two Fe-rich soils and $FeSO_4$ reduced Cu absorption by approximately 50% whereas the Fe-poor soil had no effect, suggesting that soil Fe might be important. However, in Expt 2 $FeSO_4$ retained its inhibitory effect on Cu absorption but the two Fe-rich soils did not. It is, therefore, possible that factors other than Fe in the clay and chalk soils interacted with dietary S to inhibit Cu absorption in Expt 1.

Natural diets probably contain sufficient S for the ingestion of such soils to reduce Cu absorption. The absorption of Cu from a silage containing 2·1 g S/kg DM fell from 0·076 to 0·049 when soil (weald silt, 100 g/kg DM) was added to it. Furthermore, the absorption of Cu from herbage is low in autumn, a time of increased soil ingestion (Suttle, 1981).

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The use of copper-containing controlled release glass for systemic supplementation of sheep with Cu. By P. R. MOORE, G. A. HALL, B. F. SANSOM, *Agriculture Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire RG16 0NN* and C. F. DRAKE, *Standard Telecommunication Laboratories, London Road, Harlow, Essex*

Twenty-four pairs of Herdwick lambs received a subcutaneous implant in the right flank of 33, 66 or 100 mg copper as one of two formulations of Cu-containing controlled release glass (CRG). The two formulations dissolved at different rates, are in <24 h (C_4) and the other in approximately 5 d (C_5). Eight lambs were untreated controls. Liver biopsies were taken 3 weeks before treatment for estimation of hepatic Cu concentrations. Pairs of sheep were then slaughtered 1, 3, 5 and 7 d after the implantation of C_4 glass and 3, 11, 19 and 28 d after the implantation of C_5 glass. Blood samples were taken before implantation, daily for 5 d after implantation and thereafter weekly until slaughter, and plasma Cu concentrations were measured. At slaughter each liver was weighed and subsamples taken for Cu estimation. Each of the sites of implantation was excised and analysed for Cu after a small sample had been taken for histological examination of any tissue reaction. A similar piece of tissue was removed from the opposite flank of the carcass and also analysed for Cu content.

The Cu released from C_4 glass disappeared rapidly from the implant site. Not more than 55% of the dose was found at the site after 24 h and <4% by 7 d. The tissue reactions were diffuse and oedematous and there appeared to be little relationship between the size of the reaction and the dose of Cu. Histological examination showed that necrosis, neutrophilia and granulation increased progressively with time. On average 61% of the dose of Cu was recovered from the liver of animals given 33 mg Cu as C_4 glass and the corresponding values for the doses of 66 and 100 mg Cu were 79 and 80% respectively.

Amounts of Cu recovered from the sites of implantation of C_5 glass varied greatly; for example, after 3 d between 19 and 40% of the Cu which had been released from the glass was recovered. Subsequently, the tissue reactions tended to become pus-filled abscesses which showed varying amounts of fibrosis and granulation. Between 0 and 56% of the dose of Cu was recovered from the reaction site and the pus up to 28 d after treatment. Variable amounts of Cu were recovered from the livers of animals treated with C_5 glass; between 14 and 72% of the dose could be recovered after 3 d but after 28 d not more than 12% was recovered.

Magnesium ammonium phosphate precipitation and its significance in sheep. By R. F. E. AXFORD, A. HUGHES and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

Head & Rook (1955) reported that addition of ammonium salts to the rumen of cattle caused reductions in the urinary excretion of magnesium, indicating an interference with Mg absorption. Conflicting results by subsequent workers suggest that this effect is due to a complex action involving other factors as well.

We have investigated the possibility that Mg is precipitated as magnesium ammonium phosphate under physiological conditions in the rumen of sheep by using simple aqueous solutions, made by mixing $MgCl_2$, NH_4Cl and NaH_2PO_4, and rumen liquor in which the ammonium and phosphate concentrations had been adjusted to 40 and 30 mM respectively. The solutions were adjusted to selected pH values with 0·1 M-NaOH and left to precipitate overnight before centrifugation and analysis of the supernatant fraction. Under these conditions the Mg content of the supernatants fell with increase in ammonium or phosphate concentration, and particularly with increase in pH over the range 6·2–7·2.

The white crystalline precipitates produced from three 1 l samples of rumen liquor so treated and adjusted to pH 6·8, were examined by X-ray diffraction analysis. The major component was identified from the diffraction peaks as guanite, $MgNH_4PO_46H_2O$.

To investigate the effect of such precipitation in vivo, five sheep were given a constant ration of hay and concentrates on a 2 hourly feeding routine, providing 1800 mg/d Mg. The sheep were given sequential slow intraruminal infusions of water, urea solution (up to 40 g urea/d) and ammonium chloride solution (up to 35 g NH_4Cl/d) to vary the rumen pH levels and ammonium concentrations and thus provide a range of soluble Mg concentrations in the rumen liquor. Rumen liquor samples were aspirated three times daily, centrifuged immediately, and Mg determined in the supernatants. Urine was collected daily and the Mg excreted was determined. There were significant correlations between the mean rumen liquor Mg concentrations and the amounts of Mg excreted on the same day.

We conclude that Mg is precipitated in the rumen of the sheep as $MgNH_4PO_46H_2O$ under conditions where the pH and ammonium concentration are high, which may lead to a lowered availability of Mg in the stomach. Absorption from the small intestine is of low efficiency (Field & Munro, 1977) and may also be inhibited by a similar mechanism (Smith & McAllan, 1967) leading to reduced overall availability of Mg. Appropriate conditions for this are likely to prevail in animals grazing spring pastures and may be responsible for the development of acute hypomagnesaemia.

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Influence of dietary sulphide and tetrathiomolybdate on copper metabolism in rats. By R. A. SUNDE, A. ROSS and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The inhibition of copper metabolism by molybdenum and sulphur in ruminants may be caused by tetrathiomolybdate (MoS_4^{2-}) or related compounds which possibly are formed in the rumen (Dick *et al.* 1975; Mills *et al.* 1978). Recently Mills *et al.* (1981) showed that dietary sulphide (S^{2-}) would exacerbate the toxicity of MoS_4^{2-} in rats. We have further investigated the site(s) and nature of the apparent interaction between MoS_4^{2-} and S^{2-}.

In Expt 1, male weanling rats were fed an albumin-based (200 g/kg) diet alone or with 1 μg Mo/g as MoS_4^{2-}, and with 0, 96 or 288 μg/g S^{2-} as CaS in a 2 × 3 factorial design. The diets were supplemented with 3 μg Cu/g as $CuSO_4$. After 6 weeks, MoS_4^{2-} alone had little or no effect on plasma and liver Cu concentrations and on Cu-dependent enzyme activities. S^{2-} alone caused 15–20% reductions in plasma Cu and caeruloplasmin activity. Increasing dietary S^{2-} in combination with MoS_4^{2-}, however, dramatically decreased plasma and liver Cu, plasma caeruloplasmin and erythrocyte superoxide dismutase activities, but did not decrease liver cytochrome c oxidase activity. TCA-soluble plasma Cu was reduced 20% by the combined treatments.

The same dietary treatments were used in Expt 2 and also included Cu-deficient groups given each level of S^{2-}. After 7 weeks, the rats were meal-fed 25 μCi $^{64}CuCl_2$ (4 μg Cu) and 20 μCi $Na_2{}^{35}S$ (77 μg S) in their respective diets, and killed 5 h later. Increasing dietary S^{2-} decreased ^{64}Cu absorption (from 32 to 15 to 9%). MoS_4^{2-} further decreased ^{64}Cu absorption at each level of S^{2-} supplementation (to 21, to 8 and to 6% respectively). There was, however, no significant interaction between MoS_4^{2-} and S^{2-} treatments on ^{64}Cu absorption. In Cu-deficient rats, S^{2-} reduced ^{64}Cu absorption to 2% demonstrating the potent ability of S^{2-} to impair Cu absorption when Cu is limiting. The retention of absorbed ^{64}Cu in the blood was doubled by MoS_4^{2-} but unaffected by S^{2-}. MoS_4^{2-} decreased ^{64}Cu retention in liver only when S^{2-} was fed concurrently. ^{35}S absorption and retention were only affected by S^{2-} administration and there was no indication of a $MoS_4^{2-} \times S^{2-}$ interaction.

These experiments showed that dietary MoS_4^{2-} at 1 μg Mo/g reduced tissue Cu content if S^{2-} was fed concurrently to the rats. The results demonstrated that the primary effect of S^{2-} was to decrease Cu absorption, whereas MoS_4^{2-} both decreased Cu absorption and altered the distribution of retained Cu within the body. There was no evidence of any effect of MoS_4^{2-} on S^{2-} metabolism nor was there evidence of a direct interaction between MoS_4^{2-} and S^{2-} on Cu metabolism.

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The effect of level of feeding on the rate of outflow of sodium dichromate-treated protein supplements from the rumen of dairy cows. By M. E. ELIMAM and E. R. Ørskov, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Recent advances in protein nutrition in ruminants have demonstrated the importance of the rate of outflow of protein supplements from the rumen. In many experiments, the expected increase in milk yield of dairy cows in response to feeding proteins of low degradability, was not observed when milk yield was high and it has been suggested that this could be due to an increase in the outflow of protein supplements from the rumen at high feed intakes which would lead to an overestimate of the effective degradability (Ørskov *et al.* 1980).

The experiment described here was designed to determine the effect of feed intake on rate of outflow of protein supplements treated with sodium dichromate (see Elimam & Ørskov, 1981*b*). Four Friesian cows in mid lactation were given a completely mixed hay and concentrate diet (50:50) at four feeding levels: 1·5, 2, 2·5 and 3 times the estimated energy requirements for maintenance (M) according to a 4×4 Latin Square design. Cr-treated fish meal was used, faeces were sampled and analysed for Cr and the values obtained used to estimate fractional rates of outflow of fish meal from the rumen according to the method described by Elimam & Ørskov (1981*a*).

The rate of outflow of Cr increased as feeding level increased up to 2·5 M, and then levelled out, but the over-all relationship between feeding level and mean fractional rate of outflow from the rumen was linear ($P<0.01$, see Table).

Feeding level (× maintenance requirements)	Feed intake (kg DM/d)	Mean fraction rates of Cr outflow
1·5	8·5	0·065
2·0	11·4	0·072
2·5	14·2	0·091
3·0	17·0	0·088
SE		0·005

The effect of feeding level on rates of outflow from the rumen of Cr in protein supplements was similar to that observed in sheep by Elimam & Ørskov (1981*b*), although the outflow rates were considerably greater here presumably due to the relatively higher feeding levels achieved in the dairy cows. Since the effective degradability of a protein can be greatly affected by the rate of outflow of that protein from the rumen (Ørskov & McDonald, 1979) the results here emphasize the importance of correcting for the rate of outflow according to the level of feeding.

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