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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A meeting of the Nutrition Society (One Hundred and Eighty-second of the Scottish Group) was held in the Fraser Noble Building, Aberdeen University, on Tuesday, Wednesday and Thursday, 26–28 September 1989, when the following papers were read.

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Seasonal child malnutrition in Gilan, Iran: the role of maternal work load and sedative drugs. By F. RABIEE and G. GEISSLER, *Department of Food and Nutritional Sciences, King's College, University of London, Campden Hill Road, London W8 7AH*

A detailed study was carried out in 148 families in a rural area of Gilan over three seasons in 1981–82 to assess whether, as in other agrarian communities (Annagers, 1973; Chan *et al.* 1979; Brown *et al.* 1982), seasonal variations exist in the pattern of morbidity, food availability, women's work load and the nutritional status of children.

Although there was seasonal variation in family food availability, the energy available was adequate, but non-breast-fed children under 2·5 years old had similarly inadequate energy intakes over all three seasons (70% recommended daily intake (RDI) (World Health Organization, 1973)). However, nutritional status in this age group improved significantly in autumn (October) compared with summer (June) (<90% standard weight for height (wt/ht) (National Center for Health Statistics, 1976): 22% *v.* 32%, $P<0·001$). No anthropometric data were collected in winter (February).

A higher prevalence of diarrhoea and higher maternal work load in summer were important factors in the reduced nutritional status in summer. Children with more than two episodes of diarrhoea per month had lower energy intakes and a higher percentage were wasted and stunted than those without diarrhoea. The incidence of diarrhoea was more common and severe in children whose mothers were away for more than 4 h/d, suggesting that lack of maternal supervision is an underlying factor. During the intensive agricultural work (June–August), women give sleeping drugs, mostly opium and Phenergan syrup, to their young children in order to make them sleep when they are away. The Table shows that in summer, children under 2·5 years given sedative drugs had significantly lower energy intakes and a higher proportion were wasted and stunted than those not given the drug, suggesting that the use of drugs had an important detrimental effect on the nutritional status of young children. This implies that seasonal child care facilities could help alleviate malnutrition.

	Daily energy intake						Nutritional status		
	MJ		kcal				<90% wt/ht (%)	<95% ht/age (%)	
	Mean	SD	Mean	SD	%RDI	*n*			*n*
Sedative	3·13	1·22	748	292	58	22	35	46	37
No sedative	3·72*	0·78	890*	190	69	72	30	38	89

* $P<0·05$.

Annagers, J. F. (1973). *Ecology of Food and Nutrition* **2,** 251–257.
Brown, K. H., Black, R. E. & Becker, S. (1982). *American Journal of Clinical Nutrition* **36,** 303–313.
Chan, C., Chowdhury, A. & Huffman, S. L. (1979). *Ecology of Food and Nutrition* **8,** 175–187.
National Center for Health Statistics (1976). *Growth Charts*. HRA 76–1120, **25(3),** 22.
World Health Organization (1973). *Energy and Protein Requirements. Technical Report Series* no. 522. Geneva: WHO.

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Urinary sulphates in children recovering from severe malnutrition. By C. A. MICHIE, *Queen Elizabeth Hospital for Children, Hackney, London* and *The Tropical Metabolism Research Unit, Kingston, Jamaica*

Severe malnutrition in children is accompanied by a particularly marked deficiency of available sulphur. This proposal rests on the measurement of low levels of inorganic sulphate excreted in the urine of such patients (Miller & Mumford, 1964). A particularly sensitive assay employing radioactive barium chloride has been used to measure inorganic sulphate (Picou & Waterlow, 1963), and in addition conjugated sulphate and sulphate bound to urinary glycosaminoglycans in the urine of severely malnourished children. Urines (24 h) were collected on admission of children to hospital, and on the attainment of expected weight for height on a refeeding regimen. Sixty-three paired samples were assayed. The intake of S on the day of urine collection was calculated from the measures of feed intake made on the ward both on admission and discharge so as to estimate S clearances. Control urines collected from six well-nourished age-matched children were assayed.

In the malnourished state there was little excretion of inorganic sulphate; three patients produced none at all. Conjugated sulphate is excreted at higher levels, of up to 0·5 μmol/kg per d (controls 0·5–0·8 μmol/kg per d). Excreted proteoglycan showed a lower degree of sulphation than that in control children, with 10–20 μg sulphate/mg of chondroitin sulphate at admission, and 20–30 μg sulphate/mg chondroitin sulphate in controls. These findings were similar in both marasmus and kwashiorkor. By discharge from hospital, when the children were gaining weight rapidly, the excretion of all sulphates was increased, but remained below that of control children. The crude estimates of sulphate clearance determined from inputs and combined inorganic and esterified sulphate as output, indicated remarkable conservation of sulphate both on admission and at discharge. Forty per cent of children were in negative balance on admission, some excreting 0·3 μmol/kg per d greater than their intake. All were in positive balance by discharge but 10% were in positive balance of less than 0·001 μmol/kg per d. The sulphate balance showed no obvious relationship to diagnosis, weight, height, or rate of growth at the time of sampling. One difference was observed which related to the diagnosis: significantly more highly sulphated dermatan sulphate is excreted by kwashiorkor patients than by marasmic children or controls. This may indicate the greater degree of dermal damage and regrowth in these patients.

These results demonstrate that despite dramatic conservation of S in malnourished patients, sulphate continues to be conjugated and excreted. Such conjugation must therefore represent an important route for detoxification in the malnourished patient (Weinshilboum, 1986). It is conceivable that a shortage of available S in the diet inhibits hepatic detoxification of foreign substances in the malnourished or rapidly growing child.

Miller, D. S. & Mumford, P. (1964). *Proceedings of the Nutrition Society* **23**, xliv.
Picou, D. & Waterlow, J. (1963). *Nature* **197**, 1103.
Weinshilboum, R. M. (1986). *Federation Proceedings* **45**, 2220–2228.

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Ethnic and geographic variation in growth of Hong Kong Chinese infants. By S. S. F. LEUNG, S. LUI and D. P. DAVIES, *Department of Paediatrics, Chinese University of Hong Kong, Shatin, Hong Kong*

The auxological status of 175 full-term, healthy, well-nourished infants was studied over a 2-year period, from 1984 to 1986, using standard methods, every 2 months in the first year and every 3 months in the second. The median growth curves of weight, length and head circumference were close to those of Japanese infants measured in 1980. Compared with the National Center for Health Statistics (1976) median, the mean weight curve described a more rapid rise in the first 3 months, followed by a deceleration. This shape is similar to the one described for Cambridge infants. The mean triceps and subscapular skinfold thicknesses (Table) were similar to those described in Cambridge, Germany and Canada, showing an earlier peak at 3 months, rather than at 9 months, and gradually returning to a position much lower than the Tanner's reference for skinfold thicknesses. The mean curve for mid-arm circumference described an earlier rise in the first 3 months and then slowed down to a position lower than the Wolanski's reference.

Measurements of triceps and subscapular skinfold thickness (mm)

Age (months)	Triceps				Subscapular			
	Girls		Boys		Girls		Boys	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0·25	5·6	1·0	5·5	1·0	5·1	0·9	4·9	0·9
2	9·4	1·2	9·6	1·4	7·6	1·3	7·7	1·5
4	9·6	1·4	9·4	1·5	7·5	1·4	7·2	1·2
6	8·8	1·3	8·5	1·5	7·0	1·3	6·7	1·3
8	7·8	1·4	7·6	1·5	6·5	1·1	6·3	1·3
10	7·1	1·3	7·2	1·3	6·1	1·2	5·9	1·1
12	6·9	1·3	6·9	1·3	5·9	1·1	5·7	0·9
15	5·9	1·0	6·8	1·0	5·9	1·0	5·7	0·8
18	6·8	1·4	6·7	1·2	6·0	1·0	5·7	0·8
21	6·5	1·1	6·4	1·1	5·5	0·9	5·3	0·8
24	6·3	1·1	6·1	1·0	5·5	1·0	5·2	0·8

In conclusion, the pattern of growth of the Hong Kong Chinese infants appears to be more rapid in the first 3–4 months when compared with the commonly referred National Center for Health Statistics and Tanner's reference data, and is followed by a deceleration of growth after 4 months of age. This pattern of growth is more similar to recent studies in the UK (Cambridge), Germany, Canada and Japan than to those commonly used in international references. In our early publications, we showed that these infants are well nourished (Leung *et al.*1988*a*,*b*, 1989). It is for this reason that we believe a reference curve for S.E. Asia is required.

Leung, S. S. F., Lui, S. & Davies, D. P. (1988*a*). *Australian Paediatric Journal* **24,** 186–190.
Leung, S. S. F., Davies, D. P., Lui, S., Lo, L., Yuen, P. & Swaminathan, R. (1988*b*). *Journal of Tropical Paediatrics* **34,** 100–103.
Leung, S. S. F., Lui, S. & Swaminathan, R. (1989). *Acta Paediatrica Scandinavica* **78,** 303–306.
National Center for Health Statistics (1976). *Growth Charts*. HRA 76–1120, **25(3),** 22.

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Habitual exercise patterns and daily energy expenditure. By T. J. HORTON and C. A. GEISSLER, *Department of Food and Nutritional Sciences, King's College, University of London, Campden Hill Road, London W8 7AH*

Athletes have been shown to have both a greater (Tremblay *et al.* 1985) and a similar (Poehlman *et al.* 1985) resting metabolic rate compared with their sedentary counterparts. In addition, the level of dietary-induced thermogenesis in highly active individuals has been reported to be reduced compared with inactive people (LeBlanc *et al.* 1984). The effects of regular, long-term activity patterns on total daily energy expenditure (EE) was therefore investigated.

Male subjects were selected and classified as sedentary (S), moderately active (M) and highly active (H) (ten in each group) based on habitual exercise patterns. Groups were closely matched for age, height and weight. EE (24 h) was measured in a room respirometer on two separate days: a sedentary day and an exercise day. The activity on the exercise day included three × 30 min sessions of exercise, 15 min performing step tests (24 steps/min) and 15 min cycling (20 rpm, 7% impedence).

The results for the mean 24 h EE values in absolute terms and in terms of lean body mass (LBM) are shown in the Table.

		Absolute 24 h EE (MJ/d)			24 h EE/kg LBM per h (kJ)		
Group . . .		S	M	H	S	M	H
Sedentary day	Mean	8·63	9·28	9·86*	5·89	6·23	6·23
	SD	0·71	1·12	1·09	0·50	0·92	0·63
Exercise day	Mean	11·01	11·55	11·86	7·48	7·69	7·48
	SD	1·10	1·03	1·24	0·88	0·84	0·54

H *v.* S: * $P<0·05$.

All three groups spent the majority of time in sedentary occupations on both days, except for the set exercise. Food intake over the 24 h period was based on habitual energy intake of subjects; average values were 11·40, 12·63 and 15·67 MJ for the S, M and H groups respectively. However, net thermogenesis (24 h EE −sleeping metabolic rate −net energy cost of exercise) was not significantly different between the three groups (2·28, 2·56 and 2·42 MJ on the sedentary day and 2·34, 2·60 and 2·77 MJ on the exercise day for the S, M and H groups respectively). Sleeping metabolic rate was used as an approximation of basal metabolic rate.

In absolute terms, 24 h EE was greatest in the highly active and then the moderately active group compared with the sedentary group. Differences were smaller on the exercise day. However, expressing the results in terms of LBM reduced the differences between the three groups on both days.

In conclusion, the greater daily EE of the highly active and moderately active groups compared with the sedentary group was due to their greater LBM. Exercise therefore may be of value in the maintenance of energy balance due to its effect on LBM and the consequent rise in daily EE even on sedentary days.

LeBlanc, J., Diamond, P., Cote, J. & Labrie, A. (1984). *Journal of Applied Physiology* **56**, 772–776.
Poehlman, E. T., Despres, J. P., Bessette, H., Fontaine, E., Tremblay, A. & Bouchard, C. (1985). *Medicine and Science in Sports Exercise* **17**, 689–694.
Tremblay, A., Fontaine, E. & Nadeau, A. (1985). *Canadian Journal of Physiology and Pharmacology* **63**, 1165–1169.

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The effect of ephedrine and aspirin on the metabolic rate of lean and obese women. By T. J. HORTON and C. A. GEISSLER, *Department of Food and Nutritional Sciences, King's College, University of London, Campden Hill Road, London W8 7AH*

Ephedrine has been found to enhance the metabolic rate (MR) of humans both in acute studies (Evans & Miller, 1977; Morgan *et al.* 1982) and with long-term administration (Astrup *et al.* 1986). In animals, the thermogenic properties of this sympathomimetic compound have been shown to be enhanced by the addition of aspirin (Dulloo & Miller, 1987). The effect of the combination of ephedrine and aspirin on the acute MR of obese and lean women was therefore investigated.

Ten lean and ten obese females completed each of the following treatments: (a) 1045 kJ liquid meal (M), (b) meal plus 30 mg ephedrine hydrochloride (ME), (c) meal plus 30 mg ephedrine hydrochloride and 300 mg aspirin (MEA). MR was measured using the Douglas bag technique. Three to four baseline resting MR readings (PRE) were made followed by eight post-treatment measurements over a period of 160 min (POST).

The Table summarizes the results showing the mean absolute values for MR pre- and post-treatment and the mean percentage rise in MR over 160 min post-treatment.

Metabolic rate (kJ/min)		M		ME		MEA	
		Lean	Obese	Lean	Obese	Lean	Obese
PRE	Mean	4·31	5·02	4·22	4·89	4·31	4·39
	SD	0·33	0·54	0·46	0·71	0·46	0·63
POST	Mean	5·02	5·56	5·10	5·64	5·27	5·89
	SD	0·38	0·58	0·54	0·84	0·75	0·67
Increase (%)	Mean	16·8§	11·4	20·6†§	16·1*	21·6*	20·1**‡
	SD	3·2	4·5	5·1	5·3	6·6	4·7
Absolute increase (kJ/min)	Mean	0·71	0·58	0·88	0·79	0·96	0·96**
	SD	0·12	0·21	0·21	0·25	0·33	0·17

ME/MEA *v.* M: † $P<0·06$, * $P<0·05$, ** $P<0·001$ (ANOVA).
MEA *v.* ME: ‡ $P<0·05$.
Lean *v.* obese: § $P<0·05$.

The reduced meal response of the obese group compared with the lean was corrected by the addition of ephedrine to the treatment regimen. Ephedrine increased the response in both groups but by more in the obese than the lean; however, the overall response was still less in the obese. Aspirin further enhanced the MR in the obese but not in the lean so that the effect of the combined treatment (MEA) was the same in both groups. Aspirin therefore potentiates the effect of ephedrine selectively in the obese and the combination of these two drugs has a potential role in the treatment of obesity.

Astrup, A., Madsen, J., Holst, J. J. & Christensen, N. J. (1986). *Metabolism* **35**, 260–265.
Dulloo, A. S. & Miller, D. S. (1987). *American Journal of Clinical Nutrition* **45**, 564–569.
Evans, E. & Miller, D. S. (1977). *Proceedings of the Nutrition Society* **36**, 136A.
Morgan, J. B., York, D. A., Wasilewska, A. & Portman, J. (1982). *British Journal of Nutrition* **47**, 21–32.

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Energy and nutrient intakes during high-altitude acclimatization. By C. E. FENN, S. M. DIXON and L. S. WHITTINGTON, *Robert Gordon's Institute of Technology, Queen's Road, Aberdeen AB9 2PG*

Success of a high-altitude mountaineering expedition may be limited by weather conditions but also by the nutritional status of the expedition members. A recent report has calculated energy and nutrient intakes from the use of tinned and dehydrated rations consumed during the later stages of an expedition (Lynch & Cullen, 1988). There is less data regarding energy and nutrient intakes during the initial walk-in period when the diet is based on locally available fresh food supplies.

Ten healthy unacclimatized male subjects walked from an altitude of 2430 m to Everest base camp (5400 m) in 10 d. The average increase in altitude was 300 m/d. All food and fluids consumed during the 10 d walk-in period were weighed using digital dietary scales and recorded in food record books. The diet during this time was based largely on fresh, locally available Nepalese foods such as potatoes, onions, cabbage, buffalo, goat and yak meat, dahl, rice, barlottie beans and flour. Samples of dry or non-perishable foods were collected and subsequently analysed for protein (Kjeldahl), fat (Soxtec) and carbohydrate (Dubois, 1956). Energy values of the analysed foods were calculated using the Atwater factors (17, 37, 16 kJ/g for protein, fat and carbohydrate respectively). The mean daily energy intake was 10·03 (SE 1·26) MJ. Once an altitude of 3475 m had been reached there was a significant decrease in percentage energy from fat (36 (SE 2)% on day 3 to 27 (SE 1)% on day 10) with a corresponding increase in energy from carbohydrate (46 (SE 4)% on day 3 to 61 (SE 1)% on day 10, $P<0·001$). Mean daily intakes of vitamins and minerals are given in the Table.

	Mean	SE	RDA
Thiamin (mg)	1·3	0·2	1·3
Riboflavin (mg)	1·7	0·3	1·6
Niacin (mg)	37	6	18
Folic acid (μg)	147	15	300
Ascorbic acid (mg)	37	1	30
Iron (mg)	13	1	10
Zinc (mg)	9	1	—

RDA, recommended daily amount (Department of Health and Social Security, 1979).

The RDA values refer to healthy populations but not to those exposed to high-altitude when nutrient requirements may be increased. The results indicate that, if nutrient deficiencies are to be prevented, a vitamin and mineral supplement may be beneficial.

The support of Seager Wedo (UK) Ltd is gratefully acknowledged.

Department of Health and Social Security (1979). Recommended daily amounts of food energy and nutrients for groups of people in the UK. *Report on Health and Social Subjects* no. 15. London: H.M. Stationery Office.

Dubois, A. (1956). *Analytical Chemistry* **28**, 350–356.

Lynch, P. M. & Cullen, R. (1988). *New Zealand Journal of Sports Medicine* **16**, 59–61.

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Baseline measurements for stable isotope studies. By S. D. HEYS[1,2], M. A. MCNURLAN[1], K. G. M. PARK[1,2], E. MILNE[1] and P. J. GARLICK[1], [1]*Rowett Research Institute, Bucksburn, Aberdeen* and [2]*Department of Surgery, University of Aberdeen, Aberdeen*

Stable isotopes, particularly carbon-13, have been increasingly used in clinical studies of protein synthesis in individual human tissues that can be sampled by biopsy (Garlick *et al.* 1989; Heys *et al.* 1989). However, ^{13}C is relatively abundant naturally, at a level of about 1% of the total carbon in tissues. This varies between individuals and thus it is necessary to measure the baseline enrichment of the amino acid in protein, in addition to the value after administration of isotope. In previous studies it has therefore been necessary to take a tissue biopsy both before and at the end of the labelling period (Halliday *et al.* 1988). The present study was designed to determine whether protein isolated from plasma could be used to determine the baseline enrichment in muscle or tumour tissue for individual subjects, without the need for tissue biopsy.

Patients studied were admitted to hospital for elective repair of inguinal hernia or for treatment of colorectal carcinoma. They were all from the Aberdeen area and had been eating a mixed diet, without any dietary intervention. A 10 ml venous blood sample was taken for measurement of leucine enrichment in plasma proteins, and biopsies of tumour and muscle taken immediately after induction of anaesthesia but before surgery commenced. Leucine was isolated from tissue protein by preparative ion-exchange chromatography and the ^{13}C enrichment determined by gas-isotope ratio mass spectrometry.

The ^{13}C enrichments ($\delta^{13}C_{PDB}$) for leucine in the tissues of patients with inguinal hernia were −27·15 (SEM 0·483) for plasma and −26·42 (SEM 0·339) for muscle. The results from the patients with colorectal cancer were −27·15 (SEM 0·563) for plasma, −26·77 (SEM 0·674) for muscle and −27·33 (SEM 0·519) for tumour tissue. The mean difference between plasma and muscle enrichment for all patients was 0·53 (SEM 0·18), and between plasma and tumour 0·19 (SEM 0·16).

A plasma protein sample can therefore be used to evaluate baseline [^{13}C]leucine enrichment in tumour tissue. In muscle, the difference between plasma and muscle enrichment, although significant, was small (0·53 (SEM 0·18)) and is less than 5% of the change in enrichment when labelled amino acids are given, suggesting that plasma can also be used in place of a baseline muscle sample. This provides an alternative to multiple biopsies of these tissues which may prove stressful or not possible in certain circumstances.

Garlick, P. J., Wernerman, J., McNurlan, M. A., Essen, P., Lobley, G. E., Milne, E., Calder, G. A. & Vinnars, E. (1989). *Clinical Science* **77**, 329–336.

Halliday, D., Pacy, P. J., Cheng, K. N., Dworzak, F., Gibson, J. N. A. & Rennie, M. J. (1988). *Clinical Science* **74**, 237–240.

Heys, S. D., Keenan, R. A., Wernerman, J., McNurlan, M. A., Milne, E., Calder, A. G., Buchan, V., Eremin, O. & Garlick, P. J. (1989). *Proceedings of the Nutrition Society* **48**, 101A.

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The bone mineral content of Gambian and Cambridge women. By ANN PRENTICE, J. SHAW, M. A. LASKEY, T. J. COLE and D. R. FRASER, *MRC Dunn Nutrition Unit, Cambridge CB4 1XJ* and *Keneba, The Gambia*

It is commonly believed that women with the lowest bone mineral content in the years preceding the menopause are at the greatest risk of fractures in later life due to osteoporosis. The incidence of osteoporotic fractures varies considerably worldwide, being highest in northern Europe and the USA, and lowest in Africa and the Far East. The present study was designed to compare the bone mineral content of women from two communities (Cambridge and rural West Africa) with differing fracture risk.

The investigation involved 415 Gambian women (20–86 years of age) and 323 Cambridge women (20–97 years of age). The mineral content of the radius was measured at the 1/3 distal site using single photon absorptiometry (Norland, Model 278A). This technique determines the attenuation of a fine beam of ^{125}I radiation as it traverses the bone of interest, providing a measure of bone mineral content (BMC). The radiation dose incurred is small: 20 μSv per scan. The width of the bone at the measurement site (BW) is also determined permitting the computation of BMC:BW, a commonly used index which partially standardizes BMC for differences in bone size. The BMC, BW and BMC:BW of each subject was measured in triplicate.

	The Gambia					Cambridge				
		BMC (g/cm)		BMC:BW (g/cm²)			BMC (g/cm)		BMC:BW (g/cm²)	
Age (years)	*n*	Mean	SE	Mean	SE	*n*	Mean	SE	Mean	SE
20–24	80	0·837	0·010	0·716	0·007	25	0·862	0·019	0·700	0·011
25–29	51	0·857*	0·015	0·726	0·009	34	0·902	0·020	0·723	0·008
30–34	45	0·868	0·016	0·727	0·009	31	0·859	0·017	0·719	0·009
35–39	65	0·867	0·015	0·730	0·010	20	0·911	0·018	0·725	0·011
40–44	45	0·894	0·017	0·716	0·012	29	0·891	0·017	0·718	0·009
45–49	28	0·837	0·022	0·704	0·018	51	0·861	0·015	0·705	0·008
50–54	26	0·796*	0·027	0·659	0·018	37	0·863	0·019	0·697	0·010
55–59	36	0·736	0·022	0·615	0·016	23	0·794	0·021	0·648	0·010
60–64	14	0·686*	0·028	0·562	0·023	19	0·763	0·025	0·601	0·019
65–69	8	0·579*	0·026	0·467*	0·021	16	0·720	0·035	0·575	0·023
70+	17	0·612	0·035	0·479	0·021	49	0·642	0·015	0·513	0·012

Significance of *t* test comparing Cambridge and The Gambia: * $P<0·05$.

The Table gives the BMC and BMC:BW values for Gambian and Cambridge women by age. The changes with age were similar in the two communities with values at their highest between 25 and 45 years, decreasing sharply after 45 and levelling off after 70 years. At all ages there was a tendency for Gambian women to have lower BMC values than those in Cambridge, but this was only significant in older women. This difference reflected the smaller bone widths of the Gambians; BMC:BW values were identical in the two communities for women 20–50 years of age and the differences in mineral content in older women were diminished. Contrary to expectations, this preliminary study has shown that a group of women at low risk of osteoporotic fracture have similar, or slightly lower, bone mineral contents in early adult life and after the menopause than women from a high risk area.

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Fermentation of wheat bran or sugar-beet fibre by human colonic bacteria growing in vitro in semi-continuous culture. By CORINNE J. RUMNEY and C. HENDERSON, *School of Food and Consumer Studies, Robert Gordon's Institute of Technology, Queen's Road, Aberdeen AB9 2PG* and C. S. STEWART, *Nutrition Division, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

A fermenter, similar to that devised by Miller & Wolin (1981), was supplied with a complex medium in which the major energy source was dietary fibre (10 g/l). Amino acids were also provided in the medium, as Trypticase (1·5 g/l).

The fermenter was inoculated with freshly voided human faeces, and the medium was added in six daily feeds at 3-hourly intervals between 09.00 and 24.00 hours. The feed volume was 100 ml, giving an average dilution rate of 0·036/h. Each experiment was continued for 14 d.

The sources of dietary fibre supplied were either wheat bran, extracted with methanol as described by Cummings *et al.* (1978) followed by digestion with α-amylase and amyloglucosidase to remove residues of starch (Expts A and B), or sugar-beet fibre (a residue of sugar-beet refining, low in soluble sugars) (Expts C and D).

Judged by volatile fatty acid (VFA) production and culture pH, the fermenter stabilized in the first 4 d in Expts A and B but 6 d were necessary before a stable fermentation was achieved in Expts C and D. There was a much greater VFA production in the experiments using sugar-beet fibre, indicating that the dietary fibre was more extensively used than that of wheat bran. The additional VFA was largely due to an increased production of acetate. Methane was not produced in any of the experiments.

VFA production (mmol/l per d), ammonia production (mg N/l per d) and nitrate reduction (mg N/l per d) in the fermenter

Expt		Total VFA	Acetate	Propionate	Butyrate	Ammonia produced	Nitrate reduced
A	Mean	40·20	24·52	6·61	6·44	402·56	91·90
	SD	7·08	7·14	1·53	1·53		
B	Mean	38·84	24·64	4·89	7·86	453·3	96·26
	SD	6·31	4·49	1·40	2·28		
C	Mean	83·55	65·72	8·88	7·59	313·46	104·98
	SD	20·75	18·08	3·51	2·26		
D	Mean	88·26	71·32	7·37	8·90	269·34	107·26
	SD	19·59	16·59	1·83	2·95		

The fermentation of fibre was accompanied by an accumulation of ammonia which would be derived from amino acid fermentation and urea hydrolysis. Urea in the medium would provide up to 180 mg nitrogen/l per d to the fermenter. Some of the ammonia could be formed from nitrate reduction which was rapid (Table) and did not lead to significant nitrite accumulation. The net ammonia accumulation is the resultant of these productions and the use of ammonia in bacterial amino acid synthesis from carbohydrate.

In these experiments the presence of highly fermentable fibre in the sugar-beet residue led to lower ammonia accumulations in the fermenter.

The sugar-beet fibre was a gift of the British Sugar Corporation.

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Observation of anaerobic fungi in the ruminant intestine. By E. GRENET[1], G. FONTY[2], J. JAMOT[1] and F. BONNEMOY[2], [1]*Unité Ingestion, Station de Recherches sur la Nutrition des Herbivores* and [2]*Laboratoire de Microbiologie, INRA, Centre de Recherches de Clermont-Ferrand/Theix, 63122, Ceyrat, France*

Anaerobic fungi were only recently observed in the rumen (Orpin, 1975) and the faeces (Lowe *et al.* 1987) of ruminants. They have also been seen in the intestine (Grenet *et al.* 1989) but have not been studied in any detail in this organ.

In the present experiment we observed the presence of anaerobic fungi in the intestines of three cows, each with a fistulated rumen, duodenum and caecum. The animals were fed 10 kg dry matter successively as five different diets: lucerne (*Medicago sativa*) hay, lucerne hay + beet (50:50), lucerne hay + whey (40:60), maize silage + monensin (40 mg/kg dry matter intake, DMI), and lucerne hay + monensin (80 mg/kg DMI). The monensin was administered through the ruminal cannula once a day, 1 h after feeding.

Zoospore counts were made in roll tubes 3 h after the distribution of the meal, in 1 g of digestive content; the counts varied according to the diet (Table).

Zoospore counts/g at different sites of the digestive tract with the five diets studied

	Rumen		Duodenum		Caecum		Faeces	
Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Lucerne hay	11 067	1067	247	159	510	231	547	243
Lucerne hay + beet	7000	1000	285	115	1200	100	3900	600
Lucerne hay + whey	670	330	10	0	103	98	516	492
Maize silage + monensin	29 000	11 353	380	312	1933	367	5153	3948
Lucerne hay + monensin	3267	1462	33	9	77	48	290	155

There was a similar evolution in counts in the rumen, intestine and faeces. The diets can be classified as follows in decreasing order of the number of zoospores: maize silage + monensin, lucerne hay, lucerne hay + beet, lucerne hay + monensin, lucerne hay + whey. While rhizoidal-type fungi were predominant in the rumen, large numbers of non-rhizoidal-type fungi were observed in the intestine.

These results suggest that anaerobic fungi leave the rumen with the digestive contents and pass through the intestine where some of them are probably lysed and thereby absorbed by the host animal. The results also show that monensin, even in large doses, does not eliminate the fungi from the rumen.

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Rusitec: a convenient device to study the digestive activity of anaerobic rumen fungi. By M. C. HILLAIRE[1,2] and J. P. JOUANY[1], [1]*INRA, Centre de Recherches de Clermont-Ferrand/Theix, 63122, Ceyrat, France* and [2]*Université Blaise Pascal, 63170, Aubière, France*

Anaerobic rumen fungi were only recently discovered (Orpin, 1975) and their role in the microbial ecosystem is unknown. We report here a method that uses an in vitro model (Rusitec) to study the effects of anaerobic fungi in the rumen.

All materials in Rusitec, including the nylon bags for feed, were treated before use with a solution of Desogerme 3A (Laboratoires A.C.I., Lyon) diluted 1/300, and then thoroughly rinsed. Liquid and solid contents from the rumen of adult sheep were frozen at –20° for 3 d and then used to inoculate the fermenters. Freezing eliminated protozoa but did not destroy the zoospores present in the inoculum (see Table). Furthermore, the initial addition of cycloheximide (50 mg/l) to the fermenters, in order to make them fungi-free, resulted in the irreversible disappearance of all the fungi.

Influence of freezing time to rumen contents on the survival of zoospores detected according to Joblin (1981)

Freezing time (d)	1	3	60
No. of days*	3–5	3–5	6–8

* Time at the end of which the zoospores were detected in the fermenters after thawing the rumen contents.

Feeds were introduced daily into the fermenters (wheat straw + sugar-beet pulp in a ratio of 8:1, for example, as described by Hillaire *et al.* 1990) in nylon bags (50 μm mesh). A buffer solution supplemented with trace elements, urea and sodium sulphate was continuously infused at a daily rate of 0·7.

Under these conditions it is possible to compare assessments of digestion in fermenters with fungi (about 10^3 zoospores/ml) and those without fungi. This method allows the study of the effects of different factors on the population of fungi (dietary factors: energy, nitrogen and minerals; dynamic factors: dilution rate, retention time of solid feed). In fungi-free fermenters, different anaerobic rumen fungi species can be established, alone or in association, in order to study their activity in comparison with fermenters without fungi (Hillaire & Jouany, 1989).

The authors thank Dr G. Fonty for his helpful advice on the preparation of the experiment.

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Wheat straw degradation, in Rusitec, in the presence or absence of rumen anaerobic fungi. By M. C. HILLAIRE[1,2], J. P. JOUANY[1] and G. FONTY[1], [1]*INRA, Centre de Recherches de Clermont-Ferrand/Theix, 63122, Ceyrat, France* and [2]*Université Blaise Pascal, 63170, Aubière, France*

Rumen fungi colonize plant fragments in the rumen of cattle and sheep. Their ability to degrade cellulose and to metabolize carbohydrates (Orpin & Letcher, 1979; Mountford & Asher, 1983; Fonty *et al.* 1987) suggest that they play an important role in fibre digestion and may have an effect on the pattern of fermentation in the rumen.

Liquid and solid contents from the rumen of adult sheep were frozen at −20° for 3 d and used to inoculate four fermenters (Rusitec). Two fermenters received cycloheximide (50 mg/l) at the start. All fermenters received, each day, chopped (2–3 mm) wheat straw (17·95 g dry matter (DM)) and sugar-beet pulp (2·3 g DM) placed separately in nylon bags. Volatile fatty acids (VFA) and gas production were determined. Losses of wheat straw cell-wall components from nylon bags (after 48 h incubation) were measured using the method of Van Soest. Two experiments were conducted for 3 weeks under the same conditions; the data obtained were analysed by comparison of means (Student's *t* test).

	Expt 1				Expt 2			
	n	With fungi	Without fungi	SD	*n*	With fungi	Without fungi	SD
Digestion of wheat straw:*								
DM (%)	12	40·7[a]	31·9[b]	2·8	8	40·7[a]	33·4[b]	1·2
NDF–ADF (%)	6	40·2[a]	30·2[b]	2·8	4	37·9[a]	31·3[b]	1·2
ADF (%)	6	34·2[a]	22·8[b]	3·2	4	29·3[a]	20·8[b]	1·4
Digestion of beet pulp:*								
DM (%)	12	91·7[a]	89·4[a]	3·2	8	88·5[a]	90·6[a]	5·3
End-products of fermentation:								
VFA (mmol/d)	12	48·4[a]	32·6[b]	7·9	8	44·4[a]	34·6[b]	6·4
Acetate (%)	12	67·4[a]	61·3[b]	3·3	8	66·8[a]	62·7[b]	1·3
Propionate (%)	12	23·3[a]	28·9[b]	3·1	8	23·3[a]	27·3[b]	1·2
Gas (ml/d)	12	1860[a]	1120[b]	320	8	1790[a]	1290[b]	296
CO_2:CH_4	6	4[a]	7[b]	1·0	8	5·8[a]	6·4[a]	1·2

NDF, neutral-detergent fibre; ADF, acid-detergent fibre. * Estimated by losses in nylon bags for 48 h.
[a,b] For each experiment, mean values in a horizontal row with different superscript letters were significantly different: $P<0·05$.

Anaerobic rumen fungi considerably improved digestion of the wheat straw DM (+28 and 22%). This result was confirmed by the degradation of the cell-wall components, which shows that the fungi were active against hemicelluloses (+33 and 21%) and lignocellulose (+50 and 41%). In contrast, the fungi had no action against non-cellulosic cell-wall components, such as the pectic substances from sugar-beet pulp. The increase in production of VFA (+48 and 28%) and gas (+66 and 39%) in the presence of fungi was associated with a greater production of acetate at the expense of propionate and with a lower carbon dioxide:methane ratio.

These results indicate the important role of fungi in the degradation of plant cell walls and in the microbial metabolism of the rumen.

The authors thank C. Marpillat for his technical assistance.

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Rumen probiosis: the effects of addition of yeast culture (viable yeast (*Saccharomyces cerevisiae*) plus growth medium) on duodenal protein flow in wether sheep. By P. E. V. WILLIAMS, A. WALKER and J. C. MACRAE, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

Several reports indicate that addition of yeast culture (*Saccharomyces cerevisiae*) plus growth medium (5×10^9 live organisms/g; YEA-SACC Alltech Biotechnology Center, Nicholasville, Kentucky) (YC) to diets for ruminants alters rumen fermentation patterns (Newbold *et al.* 1990; Williams *et al.* 1990) and in dairy cows leads to improvements in feed intake and milk production (Williams *et al.* 1990). The effects of these alterations on nutrient absorption have not yet been quantified.

Amounts of protein passing to the small intestine and digested therein were measured in five sheep using a cross-over design. Each animal was given a pelleted, dried grass–barley:soya ration (1:1 on a dry matter (DM) basis), at a maintenance level of feeding with or without YC (4 g/d) given top dressed onto the feed in two equal quantities at 09.00 and 16.00 hours. The ration was provided continuously from belt feeders. The sheep were fitted with rumen cannulae and simple T-shaped cannulae at the proximal duodenum and the terminal ileum. Digesta flows were calculated using the dual phase markers ^{103}Ru-phenanthroline and ^{51}Cr-EDTA (Faichney, 1975) infused into the rumen. Periods with or without YC lasted for a minimum of 28 d, with measurements made over the last 10 d of each period. The effects of YC on the composition of material flowing through the small intestine and absorption therein is shown in Fig. 1.

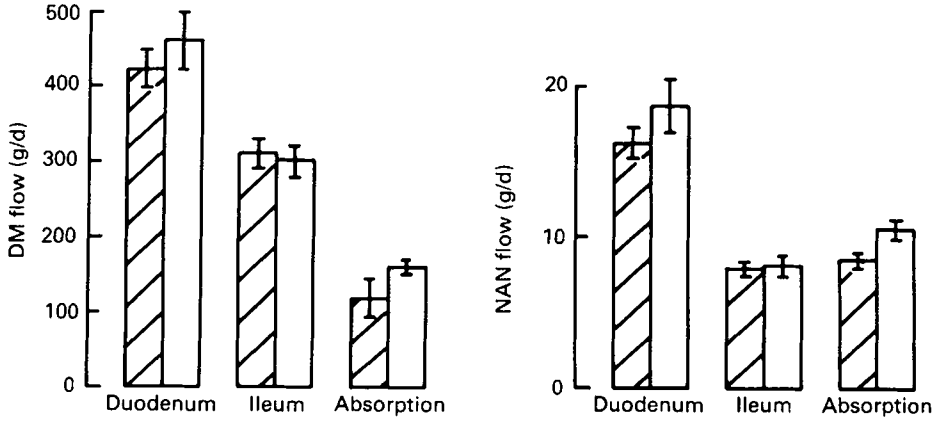

Fig. 1. Flow (g/d) of dry matter (DM) and non-ammonia nitrogen (NAN) in the duodenum and ileum, and absorption (g/d) of DM and NAN in sheep fed on diets without (□) or with (□) a top dressing of yeast culture.

The presence of YC tended to increase the flow of both DM and non-ammonia nitrogen (NAN) at the duodenum in all sheep (not significant). Addition of YC significantly increased ($P<0{\cdot}05$) the apparent absorption of DM and NAN from the small intestine.

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Some endocrine responses during heat stress induced depression of growth in young domestic fowls. By M. A. MITCHELL[1] and C. GODDARD[2], *AFRC Institutes for* [1]*Grassland and Animal Production* and [2]*Animal Physiology and Genetic Research, Roslin, Midlothian EH25 9PS*

It is well-established that chronic exposure to elevated environmental temperature reduces growth rate and feed conversion efficiency (FCE) in broiler chickens. It has been suggested that these effects are partly attributable to decreased food intake. The role of endocrine changes in the mediation of decrements in growth rate during heat stress has not been fully characterized.

In the present study groups of broiler birds (*n* 5) were exposed to temperatures of 22° or 35° for 14 d between 2 and 4 weeks of age. Food intakes and body-weights were measured daily and FCE calculated. A pair-fed group received amounts of food equal to those consumed by the high-temperature group on the previous day. At the end of the experimental period, blood samples were obtained from all birds. Each bird then received a subcutaneous injection of thyrotropin releasing hormone (TRH) (10 μg/kg) to test the function of the pituitary–thyroid axis and of stimulation of growth hormone (GH) secretion and peripheral conversion of thyroxine (T_4) to triiodothyronine (T_3) (Mitchell, 1987). Blood samples were taken at 40-min intervals post-injection. Plasma concentrations of T_4, T_3 and avian GH were determined by radioimmunoassay. Chronic heat stress reduced final body-weight by 20% ($P<0{\cdot}05$) and FCE by 17% ($P<0{\cdot}05$) compared with controls. Pair-feeding decreased only body-weight by 4%. No significant changes in plasma T_4, T_3 or GH were associated with pair-feeding, whereas heat stress induced a 63% fall in T_4 ($P<0{\cdot}05$) and a 71% reduction in T_3 ($P<0{\cdot}02$). GH levels were similar in control and pair-fed groups but slightly elevated in heat stressed birds. TRH produced similar stimulatory responses in T_4, T_3 and GH in control and pair-fed animals. TRH increased T_4 in the 35° group but the T_3 response was greatly attenuated despite an enhancement of the GH response which increased twofold.

	22° (control)		22° (pair-fed)		35°	
	Mean	SD	Mean	SD	Mean	SD
Food intake (g/d)	127·6	7·4	90·7	12·1	95·8	10·9
Wt gain (g/d)	50·7	7·4	41·2	2·5	29·9	4·7
FCE (g intake/g gain per d)	2·6	0·3	2·4	0·1	3·2	0·2
Final body-wt (g)	881	81	847	98	703	15
T_4 (ng/ml)	15·2	2·2	15·6	3·9	5·6	0·9
T_3 (ng/ml)	3·5	0·6	3·0	0·5	0·98	0·10
GH (ng/ml)	60·5	38·1	44·6	28·4	108·0	58·5

It is suggested that heat stress inhibits the stimulation of 5′-monodeiodination by GH. The resulting reduction in T_3 may at least in part contribute to decreased growth during heat stress as this hormone has been implicated in growth regulation in birds (Decuypere & Buyse, 1988).

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Effect of different dietary supplemental fats and oils on growth performance and fatty acid composition of tissues in female broilers. By J. R. SCAIFE, JESTINA MOYO, H. GALBRAITH and W. MICHIE, *School of Agriculture, 581 King Street, Aberdeen AB1 9UD*

It has been suggested that in chickens the fatty acid composition of the carcass lipids reflects that of the diet (Lipstein *et al.* 1970; Menge & Beal, 1973) and may be altered by manipulation of the dietary fatty acid intake. The present study investigated the effects of fats and oils of different dietary fatty acid composition on the growth performance and fatty acid composition of abdominal fat, liver and breast muscle of female broilers.

Female broiler chickens (360) were offered, from 19 to 54 d of age, a basal diet to which was added (50 g/kg diet): tallow (T), soya-bean oil (S), rapeseed oil (R) or marine oil (M) or 1:1 binary blends. Birds were allocated to two blocks of three-tier cages with a total of sixty cages, each containing six birds. Birds were weighed weekly and feed intake recorded. At 54 d the bird in each cage whose live weight was nearest to the average for the cage was selected for carcass analysis. Samples of liver (L), breast muscle (BM) and abdominal fat (AF) were taken for fatty acid analysis. The main effects of the inclusion of tallow, soya-bean oil, rapeseed oil and marine oil were evaluated by half-diallel analysis of variance.

Fatty acid composition (% wt of total fatty acids)

		Treatment groups											Statistical significance			
		T	T/S	T/R	T/M	S	S/R	S/M	R	R/M	M	SED	A	B	C	D
LWG (g)		1874	1888	1868	1878	1863	1883	1836	1811	1842	1797	34·8	*	*	NS	NS
FCR		2·44	2·37	2·43	2·36	2·34	2·35	2·39	2·37	2·35	2·40	0·03	*	NS	NS	NS
C18:2	L	5·95	9·12	7·94	5·19	13·2	9·69	7·56	7·90	5·85	3·89	2·44	NS	**	NS	NS
	BM	10·9	16·9	12·1	8·31	17·7	17·7	10·3	13·4	9·40	8·85	12·5	NS	**	*	**
	AF	9·47	18·3	12·9	10·0	28·5	21·1	16·5	14·8	12·9	9·42	15·4	**	**	**	**
C18:3	L	1·69	0·72	0·37	0·87	1·05	1·35	0·94	2·10	1·18	0·54	0·59	NS	NS	NS	NS
	BM	1·93	3·85	2·61	1·48	3·35	1·68	2·78	2·19	2·18	1·07	1·01	NS	*	NS	NS
	AF	1·63	2·46	2·38	1·67	4·11	3·56	2·54	3·14	1·97	1·55	0·33	**	**	**	**
PUFA†	L	5·85	8·03	7·88	4·87	5·63	6·29	5·33	5·70	6·38	6·28	2·83	NS	NS	NS	NS
	BM	5·50	6·17	5·78	15·9	8·82	8·89	12·2	5·94	12·2	5·94	1·96	**	NS	**	**
	AF	1·04	1·63	1·29	2·64	1·55	2·05	3·59	1·56	4·01	6·86	0·70	**	*	**	**

LWG, live-weight gain; A, tallow *v.* non-tallow; B, soya-bean oil *v.* non-soya-bean oil; C, rapeseed oil *v.* non-rapeseed oil; D, marine oil *v.* non-marine oil; NS, not significant.

* $P<0·05$, ** $P<0·01$.

† PUFA consists of $C_{20}+C_{22}$ polyunsaturated fatty acids.

Live-weight gain was significantly higher on those diets containing either tallow or soya-bean oil, however, only tallow showed any adverse effects on feed conversion ratio. The fatty acid composition of the abdominal fat pad was most markedly influenced by the dietary fatty acid composition. The C18:2 and $C_{20}+C_{22}$ polyunsaturated fatty acid (PUFA) contents of breast muscle were significantly higher in birds fed on diets rich in these fatty acids. It might therefore be possible to increase the unsaturated fatty acid content of the edible portion of the broiler carcass by incorporation of vegetable and marine oils into the diet.

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Differences between broiler and layer strains of chicken in the rate of adipocyte precursor proliferation. By S. C. BUTTERWITH, *AFRC Institute for Grassland and Animal Production, Poultry Department, Roslin, Midlothian EH25 9PS* and L. E. DONNELLY and A. J. CRYER, *Department of Biochemistry, University of Wales College of Cardiff, PO Box 903, Cardiff CF1 1ST* (Introduced by C. FISHER)

Although a great deal is known about the processes involved in adipocyte hypertrophy and how they are regulated, adipocyte hyperplasia has been less well studied, particularly in commercial species. We have previously shown that a broiler strain of chicken has over four times the number of adipocytes at 5 weeks of age compared with an egg-laying-strain chicken at the same age (Griffin *et al.* 1987).

Adipocyte hyperplasia reflects multiplication of adipocyte precursors rather than mature adipocytes. We have prepared stromal vascular cells from both egg-laying and broiler strains of chicken by collagenase digestion of adipose tissue. Culture and passaging of these cells leads to a homogeneous population of adipocyte precursors after the second passage (Cryer *et al.* 1987). This culture system has enabled us to compare the rates of proliferation of precursors from two strains of bird which differ markedly in their rates of adipose tissue growth. Precursor cells from broiler adipose tissue proliferated at a much greater rate than those from laying hen adipose tissue.

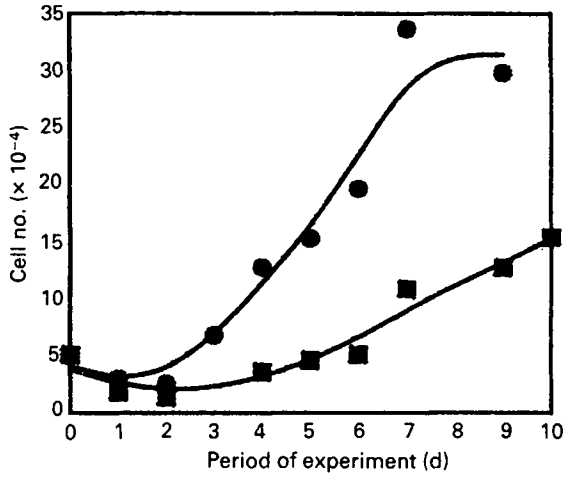

Fig. 1. Growth curves of adipocyte precursor cells from broiler (●) and laying (■) strains of chicken.

These results indicate that intrinsic cell differences between the two strains play a role in the increased hyperplasia seen in broiler adipose tissue in vivo.

L.E.D. was in receipt of an SERC studentship.

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Is brown adipose tissue present in the pig? By P. TRAYHURN[1,2], N. J. TEMPLE[1] and J. VAN AERDE[1], [1]*Nutrition and Metabolism Research Group, University of Alberta, Edmonton, Alberta, Canada T6G 2C2* and [2]*Division of Biochemical Sciences, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It is now apparent that histological appearance is not a satisfactory basis for differentiating between 'white' and 'brown' forms of adipose tissue (Trayhurn, 1989). The critical diagnostic feature of brown adipose tissue (BAT) is the presence of the tissue-specific mitochondrial uncoupling protein (UCP), which can be detected and quantified immunologically (Trayhurn, 1989). In the present study we have examined various adipose tissues from the pig for UCP, to determine whether BAT is present in this species. Although tissue with the histological appearance of BAT has been reported in 2–3-month-old animals (Dauncey *et al.* 1981), uncertainty has persisted as to whether functional BAT is present in the pig.

Pigs of the PIC breed (Pig Improvement Canada; Lacombe × Landrace with Large White) were taken at 4 d, 4 weeks and 8 weeks of age. Adipose tissue was removed from the following sites: subscapular, axillary, subcutaneous (rear back), perirenal, pericardial and peritoneal. Samples of the heart, liver and kidneys were also taken. All tissues were stored at –85°, until analysis. Mitochondria were prepared, mitochondrial proteins separated by SDS–polyacrylamide gel electrophoresis, and immunoblotting performed using a rabbit anti-(rat UCP) serum (Milner *et al.* 1989). The antibody–antigen complex was detected with goat anti-rabbit alkaline phosphatase conjugate (BioRad). Up to 25 μg mitochondrial protein were used for each sample, and the sensitivity of the immunoblotting procedure was such that 50 ng UCP was readily detectable.

No immunoreactivity at a molecular weight corresponding to UCP (32 000 daltons) was found with any of the tissues examined, both adipose and non-adipose, in pigs at either 4 d, 4 weeks or 8 weeks of age. With a view to increasing non-shivering thermogenesis and the potential for the development of BAT, 4-week-old pigs were acclimated at 10° for 10 d, but immunoreactivity consistent with UCP was again not detected in adipose tissue. Immunoreactivity corresponding to UCP was, however, evident in BAT mitochondria from several other species, including rats, golden hamsters, Richardson's ground squirrel (*Spermophilus richardsonii*) and lambs.

These results indicate that UCP is absent from adipose tissues of the pig, or is present at such a low level (<0·2% of mitochondrial protein) that it is unlikely to support thermogenesis. It is concluded that the pig does not contain adipose tissue which is functionally 'brown'.

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Insulin regulates the uncoupling protein content of brown adipose tissue. By A. GELOEN[1,2,3] and P. TRAYHURN[1,3], *Nutrition and Metabolism Research Group, University of Alberta, Edmonton, Alberta, Canada T6G 2C2,* [2]*Laboratoire de Thermoregulation et Metabolisme Energetique, URA 1341, 69373 Lyon, France* and [3]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

A role for insulin in the control of brown adipose tissue (BAT) thermogenesis has been suggested (Seydoux *et al.* 1984; Rothwell & Stock, 1988). The present study was designed to measure the effects of different peripheral levels of insulin on the uncoupling protein (UCP) content of BAT, the main index of the thermogenic capacity of the tissue.

Male ICR mice, kept at 22°, were made diabetic by a single intraperitoneal injection of streptozotocin (175 mg/kg). After 12 d of diabetes the animals received (via osmotic mini-pumps) 0, 8, 16 or 32 units insulin/kg body-weight per d. The animals were killed following insulin replacement for 12 d. Control animals were killed at the same age. Interscapular BAT was removed and protein, mitochondrial content and UCP concentration measured as previously (Milner *et al.* 1989). Results (see Table) are for ten animals or more per group.

	BAT wt (mg)		Mitochondrial protein (mg)		UCP (μg/mg mitochondrial protein)		Total tissue UCP (μg)		Blood glucose (mM)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control . . .	158·4[d]	13	8·8[d]	0·7	20·1[c]	2·3	176·7[c]	23·6	9·3[a]	0·3
Insulin (units):										
0	42·7[a]	6	3·9[a,b]	0·7	6·0[a]	1·5	26·4[a]	7·5	33·3[c]	3·6
8	65·1[a]	4	4·8[b,c]	0·4	13·3[b]	1·6	61·7[a,b]	8·3	25·5[b]	2·0
16	95·1[b]	6	5·6[c]	0·4	16·9[b,c]	1·8	96·5[b]	13·1	21·8[b]	3·0
32	129·7[c]	4	8·3[d]	0·7	15·0[b,c]	1·9	148·1[c]	26·0	9·2[a]	2·6

[a–d] Mean values in a vertical column with different superscript letters were significantly different (Fisher PLSD): $P<0·05$.

The results show that streptozotocin diabetes led to a marked fall in the concentration of UCP, and that low doses (8 units/kg per d) of insulin specifically stimulated the synthesis of the protein. Higher doses of insulin did not increase further the concentration of UCP, but markedly enhanced the mitochondrial protein content, resulting in an increased total tissue content of UCP. There was a significant correlation between the level of insulin replacement and BAT weight (r 0·73, $P<0·001$), mitochondrial protein content (r 0·68, $P<0·01$) and the total tissue UCP content (r 0·68, $P<0·001$).

These results indicate that: (1) the presence of insulin is required to maintain a normal amount of UCP, (2) insulin exerts a marked stimulatory effect on mitochondriogenesis, (3) at low concentrations insulin can specifically increase the concentration of UCP. It is concluded that the peripheral level of insulin can regulate the thermogenic capacity of BAT through the modulation of the UCP content of the tissue. Whether the action of insulin is direct, or indirect via an activation of the sympathetic nervous system, remains to be clarified.

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Use of a large dose, stable isotope procedure to measure muscle protein synthesis in sheep.

By G. E. LOBLEY, A. CONNELL, V. BUCHAN, E. MILNE, A. G. CALDER and P. A. SKENE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The large dose procedure (Garlick *et al.* 1980) has become the method of choice for measurement of tissue fractional synthesis rates (k_s) in laboratory species. The advantage of the procedure is that uncertainties about the precursor are overcome as the free amino acid pool(s) of the body are raised to a common specific radioactivity or enrichment. To date the few applications of this technique to farm animal species have involved radioisotopes and terminal procedures. A method has now been developed which utilizes the sensitivity of stable isotope procedures and the ability to biopsy, under local anaesthesia, peripheral tissues such as skin and muscle.

The technique has been applied to five wether lambs (45 kg live weight) given daily 1200 g grass pellets (10·9 MJ metabolizable energy and 30 g nitrogen/kg dry matter) at hourly intervals. The procedure involves sampling m. longissimus dorsi (200 mg by needle biopsy) and plasma protein before intravenous injection (over 8–10 min) of a mixture of 0·8 g [1^{-13}C]leucine (99 atoms %) and 4·2 g L-leucine in 250 ml water. Plasma samples are taken over the next 120 min followed by a second biopsy. Free pool plasma and muscle leucine enrichment are determined by gas chromatography mass spectrometry while protein-bound leucine is separated, decarboxylated with ninhydrin (based on Read *et al.* 1984) and measured in a gas isotope-ratio mass spectrometer.

For the sheep muscle, free leucine enrichment was 1·03 (SE 0·11) that of plasma 4-methyl-oxo-pentanoate (MOP) and the rate of decline in enrichment was 4 atoms %/h for both. Plasma MOP enrichment area was 1·03 (SE 0·02) that of plasma leucine and the rate of decline in enrichment was 4 atoms %/h for both. Initial enrichment of plasma protein exceeded that of muscle protein by 1·0 (SE 0·4)%. Mean muscle k_s was 0·0288 (SE 0·003).

The leucine injection provoked an increase in plasma insulin concentration (mU/l) of 34 (SE 6) within 15 min of the start of the injection but this had returned to baseline (20 (SE 2)) by 40 min. Because of the possible disturbance of protein metabolism through hormone release, comparisons were made of the insulin secretory activity of other amino acids in a further three sheep. Phenylalanine (3 g) was more potent when injected over 1 min compared with 8–10 min but both treatments produced a lesser reaction than leucine (5 g, 8–10 min). Valine (5 g, 8–10 min) produced no change in plasma insulin concentration. The large dose of leucine also caused decreases in the circulating concentrations of valine, isoleucine, phenylalanine and tyrosine; these had progressively declined to 40–60% of pre-injection concentration 2 h after the injection.

Use of the procedure in sequential studies could involve a different amino acid per treatment with the adoption of plasma protein to establish background enrichment.

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Measurement of tissue protein synthesis in pathological conditions of the gastrointestinal tract in man. By S. D. HEYS[1,2], K. G. M. PARK[1,2], M. A. MCNURLAN[2], R. A. KEENAN[1], J. D. B. MILLER[1], O. EREMIN[1] and P. J. GARLICK[2], [1]*Department of Surgery, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZD* and [2]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Measurements of whole body protein metabolism have previously been made in patients with cancer and inflammatory bowel disease, with increases in whole body protein synthesis and breakdown having been reported. However, there have been relatively few measurements of protein synthesis in affected tissue itself. Those studies that have been carried out, have used constant infusions of labelled amino acids, but with rapidly turning over tissues the enrichment of the precursor pool of amino acids used for protein synthesis may not be accurately determined. This problem may be reduced by using the flooding dose technique, which we have successfully applied to colorectal cancer tissue (Heys *et al.* 1989). This method has now been used to measure protein synthesis in benign colorectal tumour tissue and in colonic mucosa of patients with inflammatory bowel disease.

Patients with carcinoma of the rectum, benign tumours of the rectum and inflammatory bowel disease were studied in the post-absorptive state. They were fasted for 12 h before determination of protein synthesis by injecting 4 g L-[1^{-13}C]leucine/70 kg body-weight intravenously (20 atoms %). Biopsies of tumour tissue and mucosa were obtained immediately after induction of anaesthesia. The fractional rates of protein synthesis were calculated from the increase in L-[1^{-13}C]leucine enrichment in tissue protein and the enrichment of leucine in the plasma determined by isotope ratio and gas chromatography–mass spectrometry.

The rates of protein synthesis for each group, expressed as the proportion of the tissue protein pool renewed each day, were as follows; carcinoma of the rectum 22·5 (SEM 2·4) (*n* 6), benign rectal tumours 38·1 (SEM 2·6) (*n* 6), inflammatory bowel disease 26·8 (SEM 3·2) (*n* 5), normal colonic mucosa 9·5 (SEM 1·5) (*n* 5).

The flooding dose technique can be applied in man to rapidly turning over tissues that can be obtained by biopsy. The results demonstrate that protein synthesis is significantly higher ($P<0·05$) in tumour tissue and inflamed mucosa than in normal colonic mucosa.

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Influence of tumour necrosis factor α on blood cell and plasma metallothionein-I concentrations in rats. By I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and R. F. GRIMBLE, *Human Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Infection, inflammation and other types of stress induce synthesis of metallothionein (MT) in the liver. The cytokine, tumour necrosis factor α (TNF-α), which is released from macrophages in response to components of bacteria, also induces hepatic MT synthesis (Grimble & Bremner, 1989). Stress-induced synthesis of MT by endotoxin or turpentine is accompanied by release of the protein into plasma but MT concentrations in blood cells are unchanged or decreased (Bremner *et al.* 1987). We have now examined the effects of human recombinant TNF-α on plasma and blood cell MT-I concentrations in rats.

Male Wistar rats (154 (SE 1) g) were separately caged and fed on laboratory chow. Animals received intravenous injections of TNF-α (<0·137 ng endotoxin/mg protein) or sterile, non-pyrogenic saline (9 g sodium chloride/l) via the lateral tail vein (*n* 6). Blood was collected and the rats were killed 8 or 24 h after injection. Pair-fed, saline-injected controls were included since TNF-α reduced appetite. Blood cells were separated by centrifugation and washed once with saline. Concentrations of MT-I were measured by radioimmunoassay (Mehra & Bremner, 1983).

		Plasma MT-I (ng/ml)						Blood cell MT-I (ng/ml cells)					
Period after injection (h) . . .		0		8		24		0		8		24	
	n	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control, *ad lib.* fed	6	17	2	10	1	12	1	58	7	68	12	65	13
TNFα (300 μg/kg)	6			27**	3	32**	2			65	13	50††	6
Control, pair fed	6					22	6					93	13
TNFα (30 μg/kg)	6			34**	2	33**	3			72	3	54†	4
Control, pair fed	6					20	1					78	9

Significantly different from *ad lib.* control: ** $P<0·001$.
Significantly different from pair-fed control: † $P<0·05$, †† $P<0·001$.

TNF-α increased plasma MT-I concentrations by similar amounts at 8 and 24 h after injection and at both dose levels. However, there were no consistent increases in blood cell MT-I concentrations. TNF-α therefore has similar effects to endotoxin and other inflammatory agents on the appearance of MT-I in the circulation (Bremner *et al.* 1987).

The authors are grateful to BASF/Knoll AG, Ludwigshafen, FRG, for the gift of TNF-α.

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Changes in protein metabolism of cultured muscle cells treated with cimaterol. By J. M. M. HARPER and P. J. BUTTERY, *Department of Applied Biochemistry and Food Science, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics LE12 5RD*

The present study set out to examine whether the β-adrenergic agonist cimaterol does affect protein metabolism of muscle cells in vitro and whether such changes can be correlated with the binding of the compound to β-receptors. Two continuous rodent muscle cell lines were used: L6 (rat) and G8-1 (mouse). Cells were grown to confluence and allowed to differentiate before use. Methods for L6 cells were as described by Harper *et al.* (1987). G8-1 cells were grown in Dulbecco's Modification of Eagle's Medium containing 100 ml fetal calf serum/l; differentiation medium was 40 ml horse serum/l, 10 ml fetal calf serum/l.

In protein metabolism studies, cells were serum starved (18 h L6 cells, 4 h G8-1 cells) and then continuously treated with concentrations of cimaterol between 10 μM and 0·1 nM, in the absence of serum. Protein synthesis was measured as the incorporation of [^{3}H]tyrosine into trichloroacetic acid (50 g/l) (TCA)-insoluble material over a 6 h period. Significant increases were seen in both lines ($P<0·001$, Student's t test), with a half maximal effect at around 5 nM. Maximal stimulation was 12 (SD 4·6)% for L6 cells (three experiments), and 12% and 13% in two experiments in the G8-1 line. Protein breakdown was measured as the release of TCA-soluble material from cells, previously labelled with [^{3}H]tyrosine (Harper *et al.* 1987); unlabelled tyrosine was present at high concentration (>2 mM). An 18 h period was used in G8-1 cells and 24 h in L6 cells; no significant differences from controls were observed in either case.

The hydrophilic ligand (-)[^{3}H]-CGP-12177 was used in binding studies. Methods were modified from those of Pittman & Molinoff (1983); cells remained as monolayers throughout the equilibration and washing stages and were solubilized with 0·5 M-sodium hydroxide (37° for 2 h) for counting. Non-specific binding was measured in the presence of 10 μM-DL-propranolol. β-Receptors of similar affinity for the ligand were demonstrated in the two cell lines. Competition studies in L6 cells showed that cimaterol bound to these; its dissociation constant was 26 (SD 6·9) nM (n 5). The addition of propranolol, a β-antagonist (10 μM), blocked the effect of cimaterol (0·1 μM) on protein synthesis in L6 cells which indicates that this response may be mediated via the β-receptor.

Responses to β-agonists in vivo are probably the result of not only the compound's interaction with tissues directly, but also overall changes in circulating hormone and nutrient profiles. However, the possibility of direct anabolic responses of muscle cells must be included in any description of the total system.

The support of the AFRC is gratefully acknowledged.

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Response of sheep to cimaterol and white fishmeal supplementation of diets containing sub-maintenance levels of energy. By H. GALBRAITH, B. MINASSIE and J. R. SCAIFE, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Lean tissue and fat deposition may be altered by the β-adrenergic agonist cimaterol in growing sheep (Beermann *et al.* 1986) and by the inclusion of white fishmeal as a high-protein supplement in the diet of mature sheep offered low-energy diets (Vipond *et al.* 1989).

Twenty-four male castrate sheep, aged about 10 months and weighing 56·5 kg, were offered diets containing approximately 0·7 of the metabolizable energy requirement for maintenance, with or without a supplement of white fishmeal. The diets contained (g/kg dry matter) either 20 (L) or 80 (H) estimated rumen undegradable protein and adequate rumen degradable protein. The diets were unsupplemented (U) or supplemented (C) with cimaterol (Boehringer Ingelheim Vetmedia, Gmbh) to give individual intakes of 2·5 mg/d during the 49 d study period. The results were evaluated by analysis of variance and the significance of the main effects are shown in the Table.

		Treatment groups					Statistical significance		
		UL	CL	UH	CH	SED	Ci	Pr	Int
Initial live wt (kg)		57·1	57·1	56·1	56·7	0·90			
Live wt change (kg)		−7·6	−7·5	−5·8	−5·0	0·93		**	
Cold carcass wt (kg)		20·7	21·1	19·8	23·0	0·8	***		**
Cross-sectional area of m. longissimus dorsi (cm^2)		11·5	14·8	10·7	16·1	1·0	***		
Peri-renal and retroperitoneal fat (g)		194	110	182	102	26	***		
Plasma urea (mg/l)	(a)	222	207	314	235	21·4	***	***	*
	(b)	328	323	335	372	56·3		*	
Plasma free fatty acids (μeq/l)	(a)	1847	863	1236	727	233	***	*	
	(b)	643	815	1217	834	181		*	*
Carcass: Crude protein (kg)		3·72	4·59	3·30	5·01	0·23	***		
Fat (kg)		4·46	2·93	3·58	3·19	0·32	**		
Non-carcass: Crude protein (kg)		2·48	2·57	2·62	2·74	0·14			
Fat (kg)		1·94	1·45	1·99	1·55	0·15	**		

(a), day 9; (b), day 49; SED, standard error of the difference; Ci, cimaterol; Pr, protein; Int, interaction between Ci and Pr.

* $P<0·05$, ** $P<0·01$, *** $P<0·001$.

Cimaterol treatment on average resulted in (1) increases in carcass weight, weight of carcass crude protein (nitrogen × 6·25) and cross-sectional area of m. longissimus dorsi and (2) reductions in the weight of peri-renal and retroperitoneal fat and solvent-extractable fat in the carcass and non-carcass body components, and in concentrations of plasma urea and free fatty acids on day 9 but not on day 49. Significant effects due to fishmeal supplementation were reduced live weight loss and increased concentrations of plasma urea (days 9 and 49) and reductions in concentrations of plasma free fatty acids, on average, on day 9, and on diet H on day 49.

Significant interactions indicated that the effect of cimaterol on carcass weight, plasma urea and free fatty acid concentrations were greatest on diet H. The results suggest that cimaterol but not fishmeal was effective in maintaining or increasing protein and reducing fat deposition in sheep on the low-energy diet used.

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The effects of femoral arterial infusion of cimaterol on hind-limb metabolism in growing lambs. By J. BROWN, L. A. CROMPTON and M. A. LOMAX, *School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 2AJ*

β_2-Agonists have been shown to increase the amount of lean tissue in the carcass of farm animals (Baker *et al.* 1984). It is not known whether this is due to direct or indirect action of the drug on muscle tissue. The objective of the present study was to attempt to demonstrate direct effects of the β_2-agonist cimaterol on hind-limb metabolism in growing lambs by infusing the drug into the femoral artery.

Six wether lambs, live weight range 30–40 kg, were fed twice daily on a diet of concentrate (Lamlac start–finish, Volac Ltd, Herts) (35 g/kg body-weight per d) and chopped hay (5 g/kg body-weight per d). A growth rate of 351 (SE 15) g/d was achieved. Catheters were inserted into the carotid artery and the femoral arteries and veins of both hind-limbs. Saline (S) (9 g sodium chloride/l), a low dose of cimaterol (LC) (0·1–0·2 mg/d), or a high dose of cimaterol (HC) (2·5–2·7 mg/d) was infused into one femoral artery while saline (LS or HS) was infused simultaneously into the contralateral femoral artery as a control. Infusions lasted for 5 d and on the last day of treatment hind-limb blood flow, oxygen uptake and tyrosine uptake were measured. Samples were taken from the carotid artery and both femoral veins for a 6 h period, followed immediately by hind-limb blood flow estimation using an equilibrium diffusion method (Lindsay *et al.* 1978). The results are shown in the Table.

		Plasma flow rate (ml/min per g)		O_2 uptake (μmol/min per g)		Tyrosine uptake (nmol/min per g)	
Treatment	*n*	Mean	SEM	Mean	SEM	Mean	SEM
S	4	0·073	0·007	0·145	0·02	0·416	0·042
LS	5	0·062	0·005	0·148	0·03	0·573	0·131
LC	4	0·100**	0·003	0·225**	0·02	0·916**	0·101
HS	3	0·120**	0·006	0·299**	0·02	0·836**	0·106
HC	4	0·118**	0·008	0·351*	0·06	0·642	0·100

Significantly different from saline control: * $P<0·05$, ** $P<0·01$.

Cimaterol infusion, at a low dose, had a local direct effect on the cimaterol-infused hind-limb without any apparent recirculation effects on the saline-infused leg. Cimaterol infusion significantly increased blood flow, O_2 uptake and tyrosine uptake. In the high-dose treatment, recirculation appears to have occurred as shown by the increased values in both saline- and cimaterol-infused legs. However, at the high dose of cimaterol, tyrosine uptake was not increased in the treated leg despite increased blood flow. The tyrosine uptakes, when expressed in terms of protein gain, are larger than previously observed in carcass slaughter trials. Therefore, cimaterol has a direct effect on hind-limb blood flow, O_2 uptake and tyrosine uptake. It appears that β-receptors in the hind-limb may have different sensitivities with respect to blood flow and protein metabolism.

The authors acknowledge the gift of cimaterol from Boehringer Ingelheim. J.B. wishes to acknowledge the support of a MAFF studentship.

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Reduction–oxidation state and protein turnover in rats treated with a non-selective β-agonist. By J. A. MARTINEZ, A. S. DEL BARRIO, M. P. PORTILLO, M. J. RGUEZ-MARISCAL and J. LARRALDE, *Department of Physiology and Nutrition, University of Navarra, 31008 Pamplona, Spain*

Selective β-adrenergic agonists have been reported to increase carcass protein and decrease lipid accretion in a wide variety of species (Hanrahan, 1987). In this context, the metabolic actions mediated by β_1 binding are generally recognized as lipolytic and also antilipogenic, while β_2 receptors appear to be associated with glycogenolysis and with protein turnover. Studies conducted to date have not included mechanistic information on adrenergic compounds with both β_1 and β_2 receptor specificity. Accordingly, an experiment using a rodent model was undertaken to elucidate the action of a non-selective β-agonist on protein turnover.

Male Wistar rats (initial weight about 90 g) were fed *ad lib.* on a standard laboratory diet. The animals were injected subcutaneously twice daily (09.00 and 17.00 hours) with metaproterenol (1 mg/kg) or vehicle for 22 d. Determinations of amino acids (Martinez *et al.* 1989) and the lactate:pyruvate ratio (Fagan & Tischler, 1986) were carried out in plasma and gastrocnemius muscle, respectively.

	Control		β-Agonist		Statistical significance
	Mean	SE	Mean	SE	
Daily gain (g/d)	6·4	0·5	6·2	0·3	NS
Gastrocnemius wt (g)	1·05	0·03	1·22	0·04	$P<0·001$
Carcass protein (%)	19·6	0·2	21·0	0·5	$P<0·05$
Plasma amino acids (μmol/ml)	5·4	0·2	4·8	0·1	$P<0·01$
Cathepsin A activity (U/g)	113·4	2·8	101·1	4·6	$P<0·05$
Lactate:pyruvate ratio (mmol/μmol)	80·3	4·1	119·5	4·3	$P<0·001$

NS, not significant (Student's *t* test).

No changes in growth rate were observed in the animals on the β-agonist treatment; however, gastrocnemius weights were significantly increased in the metaproterenol group, which was accompanied by a rise in the carcass protein content, as previously reported (Portillo *et al.* 1988). The plasma amino acid concentrations were markedly reduced in the animals given the sympathomimetic compound, which is in good agreement with values obtained with other growth-promoting agents. The cathepsin A activity in the gastrocnemius muscle was lower in the β-agonist-treated rats compared with the controls, and was associated with a more reduced redox state as indicated by the lactate:pyruvate ratio.

In summary, these findings support the conclusion that the protein anabolic effect obtained with a non-selective β-adrenergic agonist involves a decrease in muscle protein breakdown accompanied by a reduction of the redox couple and a fall in plasma amino acid levels, as reported following different approaches (Hanrahan, 1987).

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Effect of lactation products on brush-border enzyme expression in sucking pigs. By D. KELLY, T. P. KING and M. MCFADYEN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Hormones and growth factors in maternal milk have been shown to regulate cellular proliferation and differentiation of the gastrointestinal tract of neonatal rats (Henning, 1987). In the present investigation the influence of milk composition on the temporal development of the porcine small intestine was examined.

Forty piglets from four sows (Large White × Landrace) were given, by gavage, approximately 40 ml standard colostrum every 3 h for 36 h postpartum, then allocated to two sucking regimens. Those in group 1 were normally suckled (N) for 8 weeks on a single dam and consequently received both early and late lactation products. Group 2 were cross-fostered (CF) weekly onto a newly farrowed sow for 8 weeks and consequently received only early lactation products. Animals were slaughtered at 3, 5, 7 and 8 weeks postpartum, and samples of tissue were taken from five sites along the small intestine. Mean values from the five sites for lactase, total glucoamylase (TGA), mucosal protein, DNA, villus height and crypt depth are given in the Table.

(Results are means of five small intestine sites)

		Weeks postpartum				SED	Statistical significance
		3	5	7	8		
Lactase (μmol/min per g protein)	N	204·5	94·4	64·6	52·2	11·3	$P<0·001$
	CF	104·5	40·5	38·0	39·0		
TGA (μmol/min per g protein)	N	19·11	41·78	39·43	47·50	3·908	$P<0·01$
	CF	18·52	25·87	30·14	34·63		
Protein (mg/g mucosa)	N	137·8	137·4	132·4	132·9	8·20	NS
	CF	155·4	143·6	140·4	136·5		
DNA (μg/g protein)	N	43·0	47·6	51·7	65·6	9·88	NS
	CF	42·8	42·1	53·7	60·7		
Villus height (μm)	N	572·8	479·8	468·3	473·2	34·95	NS
	CF	538·0	392·7	422·0	444·2		
Crypt depth (μm)	N	121·2	158·1	191·6	225·6	9·15	$P<0·05$
	CF	135·4	185·5	192·1	221·6		

NS, not significant.

Loss of brush-border lactase activity was significantly ($P<0·001$) greater in CF compared with N. Although the temporal appearance of TGA was similar in both groups the increase in enzyme activity was significantly greater ($P<0·01$) in N compared with CF. There was no significant effect on protein, DNA or villus height. Crypt depth was significantly increased ($P<0·05$) in CF pigs. The results demonstrate that lactation products can influence cellular differentiation and the expression of brush-border enzymes.

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Effect of lactation on the postnatal expression of intestinal membrane glycoconjugates in pigs. By T. P. KING and D. KELLY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In the small intestine, oligosaccharide moieties on both enzyme and non-enzyme glycoconjugates provide a wide range of potential binding sites for luminal or circulating biologically active ligands such as growth factors, bacterial adhesins, toxins and dietary lectins. The postnatal expression of intestinal glycoconjugates and their potential value as differentiation markers has not been previously investigated in pigs. The present study describes the age-related and lactation-related changes in terminal glycosylation of blood-group antigens and their precursors in the pig small intestine during sucking.

Two experimental groups of twenty piglets were placed onto two different sucking regimens. Group 1 were suckled normally (N) for 8 weeks on single dams and thereby received both early and late lactation products. Group 2 piglets were cross-fostered (CF) each week onto newly farrowed sows and thereby received only early lactation products. The experimental protocols and sampling procedures were as described by Kelly *et al.* (1990). Pigs were killed at 1, 3, 5, 7 and 8 weeks postpartum and formaldehyde-fixed intestinal tissues were embedded in histological resin. Carbohydrate-specific lectins and monoclonal antibodies, labelled with fluorescein isothiocyanate or tetramethyl rhodamine isothiocyanate, were used in high-resolution cytochemistry of blood-group and precursor oligosaccharides on microvillar and other intestinal membrane glycoconjugates.

The cytochemical analysis revealed distinctive age-related intestinal glycosylation changes during the sucking period. Membrane fucosylation and the expression of blood-group H-antigen was evident from the 5th postnatal week in the N group and from the 3rd week in the CF group. More complex glycosylation involving further fucosylation or the expression of blood-group A-antigen, or both, was evident from the 7th week in the N group and from the 5th week in the CF group. These results demonstrate that two or more glycosylation waves occur in the porcine small intestine during the sucking period and that lactation can significantly influence cellular differentiation and the temporal expression of intestinal membrane glycoconjugates in sucking pigs.

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Effects of long-term (2 year) feeding of rats on raw soya bean. By G. GRANT, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Feeding young rats on diets containing raw soya bean impairs their growth and induces pancreatic hypertrophy and hyperplasia (Liener, 1981). This is due to the presence of trypsin inhibitors, lectin and other anti-nutritional factors in the seed (Grant *et al.* 1989). The aim of the present study was to evaluate the effects of feeding these potent anti-nutrients over a prolonged period (2 years).

Inclusion of raw soya bean as the only (100 g/kg) source of protein in diets for rats considerably impaired their growth for up to 20 weeks. However, during the remainder of the 2-year period, soya-bean-fed rats gained slowly in weight at rates similar to that of the controls pair-fed on a lactalbumin-based diet. This reduction in susceptibility to the anti-nutritional effects of soya bean was not apparently due to adaptation of the rats to the diet but rather to age-related changes in body metabolism.

During the initial 16–24 weeks, the pancreas of soya-bean-fed rats became progressively enlarged compared with that of controls. Over the following 40 weeks little pancreatic growth occurred on either dietary treatment. Subsequently, the pancreas of soya-bean-fed rats rapidly increased in size, reaching on average 3·5 mg/g dry body-weight after 2 years. Indeed, the largest pancreas found accounted for 12 mg/g dry body-weight. No increase in pancreatic weight was evident during this period in controls (1 mg/g dry body-weight). During this rapid phase of growth, preneoplastic changes and pancreatic tumours were evident in 8–15% of the soya-bean-fed rats.

The number of receptors on the rat pancreas for the trophic cholecystokinin (CCK) greatly increases with age (Leung *et al.* 1986). Thus, since soya-bean-induced pancreatic enlargement is, in part, mediated through CCK (Fushiki & Iwai, 1989), the rapid growth observed after one year of soya-bean feeding may be linked to these age-related changes. Furthermore, since CCK is a potent co-carcinogen (Howatson, 1986) the rise in CCK receptor concentration may increase the susceptibility of the pancreas to neoplasia.

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***Phaseolus vulgaris* lectin (PHA) induces growth of rat small intestine.** By A. PUSZTAI[1], G. GRANT[1], S. W. B. EWEN[2], D. S. BROWN[1] and S. BARDOCZ[2], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and [2]*Department of Pathology, Aberdeen University Medical School, Foresterhill, Aberdeen AB9 2ZY*

Some plant lectins, such as the lectin from the seeds of kidney beans (PHA) or soya beans (SBL), have recently been shown to stimulate the polyamine-dependent, hyperplastic growth of rat small intestine in vivo by significantly increasing its weight and DNA, RNA and protein contents in a strictly dose- and time-dependent way (Pusztai *et al.* 1986; Oliviera *et al.* 1988). The lectin PHA introduced into the intestinal lumen binds specifically to cells of the upper part of the absorptive villi and disrupts their microvillar luminal membranes (King *et al.* 1986). The extent of lectin binding decreases towards the base of the villi (King *et al.* 1986). However, despite a virtually undetectable attachment to the crypts of the Lieberkuhn, cellular proliferation in the crypts is highly stimulated by PHA (Pusztai *et al.* 1988). In time-course studies it was shown that gut growth was preceded by a substantial rise in the amounts of tissue polyamines (Pusztai *et al.* 1988), compounds intimately involved in DNA, RNA and protein syntheses (Palmer *et al.* 1987). Thus, spermidine content doubled in less than 24 h after PHA-stimulus, well before any change in the weight of the small intestine could be observed. Also, the increase correlated well with the substantially elevated fractional rate of protein synthesis (about 75% above the basal level) in vivo after intragastric intubation of pure PHA (Pegg, 1986). However, by day 3, the weight, RNA and DNA contents of the small intestine increased significantly and, according to histological studies, significant lengthening of crypts and increase in crypt cell numbers were detected (Pusztai *et al.* 1988). Moreover, from day 3 onwards, gut growth was maintained continuously for the entire duration of dietary exposure to PHA.

The continuous growth of the small intestine on PHA-diets occurs against the background of weight loss and eventual death of the animal. Indeed, the need to maintain the integrity of the small intestine and to prevent systemic toxicity, imposes a substantial nutrient and energy cost for the animal. Approximate calculations show that on the 3rd day about 15–20%, and on the 7th day about 40% of the dietary protein intake (or equivalent amounts of body protein) are used for the requirements of the growth process. By 10–16 d, the rats are in negative nitrogen balance and the dietary protein intake cannot cover the needs of the animals. Thus, catabolism of body, mainly muscle proteins (Oliviera *et al.* 1988), is required to support the growth and integrity of the gut. These findings indicate that components of food, such as lectins, can function as growth signals for the small intestine. Through their effects on the gut, lectins are able to influence body metabolism and the use of energy and proteins of both food and the body.

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Nutritional evaluation of sweet lupinseed (*Lupinus angustifolius*). By P. YEN, G. GRANT, M. F. FULLER and A. PUSZTAI, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Lupinseed, despite its high protein content (300–400 g/kg), has been used in foods for animals and man only to a limited extent. This is due to the generally high alkaloid content of the seed (Cheeke & Schull, 1985). However, with the development of low alkaloid varieties, interest in the use of this legume seed has been renewed. The aim of the present study was to estimate the nutritional quality of low-alkaloid lupinseeds using young, rapidly growing rats.

Raw lupinseed (*L. angustifolius*) was obtained from the Grain Pool of Western Australia (Perth, Western Australia). Feeding studies with 33-d-old rats were done, as before (Grant *et al.* 1986), using 100 g protein/kg isoenergetic diets based on lupinseed or egg albumin (control).

Diet and feeding regimen . . .	Lupinseed *ad lib.*	Lupinseed + suppl.† pair fed	Control, pair fed	Lupinseed + suppl.† *ad lib.*	Control, *ad lib.*
n . . .	8	4	4	4	4
Food intake (g/rat per 10 d)					
Mean	67[a]	67[a]	67[a]	192[b]	171[c]
SE	3	1	1	7	4
Wt gain (g/rat per 10 d)					
Mean	3[a]	12[b]	17[c]	64[d]	66[d]
SE	2	1	1	3	3
Net protein utilization	30–35	70	98	53	92
Nitrogen digestibility	88–89	90	99	88	99

[a–d] Mean values in a horizontal row with different superscript letters were significantly different: $P<0{\cdot}01$.
† Supplemented with individual amino acids.

Food intake, net protein utilization and weight gain were greatly reduced by inclusion of raw lupinseed as the only source of protein in diets for rats. This was partly due to deficiencies in some of the essential amino acids. Thus, on supplementation of the lupinseed diet with methionine, valine, isoleucine, phenylalanine, tryptophan, leucine and lysine to bring the dietary levels of these amino acids up to the target requirements for rats, food intake increased almost threefold and indeed was significantly higher than that of *ad lib.*-fed controls. However, the utilization of dietary N was still well below that expected. Therefore, rats grew at slower rates than controls pair-fed on diets based on egg albumin.

This impaired utilization of lupinseed protein by rats appears to be due to its lower digestibility and to the presence of anti-nutritional factors in the seed. These are apparently not alkaloids or flatus glycosides since cold or hot aqueous ethanol extraction of the lupinseed meal before its use did not improve food intake or animal growth.

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Effects of cellulose, pectin and guar gum on gastric emptying, digestibility and absorption in resting dogs. By V. DE HAAN, L. ISTASSE, S. JAKOVLJEVIC, I. DUFRASNE and J. M. BIENFAIT, *Veterinary Faculty, 45 rue des Vétérinaires, 1070 Bruxelles, Belgium*

Dietary fibre of vegetable origin is known to modify transit time, digestibility and absorption of nutrients in different ways depending on type of fibre. Several types of fibre, varying in chemical and physical composition, have been studied. However, the supplements used are not always purified fibres but often complex feedstuffs so that it is difficult to separate the effects of the fibre *per se* (de Haan *et al.* 1989).

Four young adult beagles were used in a 4 × 4 Latin-square design. The composition of the control diet (g/kg dry matter basis) was: minced meat 389, cooked rice 472, maize oil 83 and minerals + vitamins 56. The control diet was offered to provide 550 kJ (132 kcal) metabolizable energy/kg body-weight$^{0.75}$ (National Research Council, 1985). The basal diet was then supplemented with cellulose, pectin or guar gum at a level of 35 g/kg dry matter. Gastric emptying and apparent digestibility were measured and an absorption test carried out.

Gastric emptying was determined after a barium meal by sequential radiographs taken after 5, 15, 60, 120, 180, 360, 540 and 720 min. The transit time tended to be decreased with pectin and increased with guar gum compared with the control diet, while there were no differences when cellulose was added.

The apparent digestibility coefficients are given in the Table. The dry matter contents of faeces were similar (33·5 and 35·8%) when the control and the cellulose diets were offered. They were lower when pectin and guar gum were added (26·0 and 23·8% respectively). The apparent dry matter digestibility of the control diet was over 90%. Cellulose reduced the digestibility of dry matter ($P<0·05$), pectin the digestibility of protein ($P<0·001$) and guar gum the digestibility of protein ($P<0·001$) and ether extract ($P<0·05$).

Apparent digestibility (%) of a control diet and diets with the addition of cellulose, pectin or guar gum

Diets	Dry matter	Organic matter	Ash	Crude protein	Ether extract
Control	90·76	94·58	27·36	91·71	98·49
Cellulose	87·84	91·42	27·59	89·64	98·23
Pectin	88·66	92·41	24·53	86·97	97·68
Guar gum	88·06	93·78	28·10	86·47	96·43
SED	1·16	1·50	7·92	0·63	0·56

The xylose test was used to monitor intestinal absorption in fasted animals. A xylose solution (100 g/l) was given by an intragastric tube at 0·5 g/kg body-weight. Blood was serially taken after 30, 60, 90, 120, 180, 240 and 360 min. The post-prandial plasma xylose curve was similar when the control, pectin and guar gum diets were given. In contrast, the xylose peak appeared 30 min later and was smaller ($P<0·05$ or 0·02) when the cellulose diet was given.

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Effects of cellulose, pectin and guar gum on plasma insulin and metabolites in resting dogs. By L. ISTASSE, V. DE HAAN, J. F. BECKERS, C. VAN EENAEME and J. M. BIENFAIT, *Veterinary Faculty, 45 rue des Vétérinaires, 1070 Bruxelles, Belgium*

Dietary fibre has been shown to influence transit time, digestibility and absorption of nutrients (de Haan *et al.* 1989). For these reasons, dietary fibre has been used as an aid to the dietary management of obese and diabetic subjects.

Four young adult beagles as outlined in the previous paper were used in a 4×4 Latin-square design and given the same diets as previously (de Haan *et al.* 1990).

Blood samples were taken before feeding and then 20, 40, 60, 90, 120, 180, 240, 300 and 360 min after feeding. Before feeding, mean insulin concentration was 13 μU/ml, with no significant differences between treatments. Insulin concentration remained relatively low until 3 h after feeding and then increased to a plateau of about 30 μU/ml in animals given the control and the guar gum-supplemented diets. The inclusion of pectin resulted in a peak which appeared earlier and reached a significantly ($P<0·05$) higher concentration (50 μU/ml). When cellulose was added the insulin profile was intermediate between those of animals given the control and pectin-supplemented diets.

The average fasting plasma glucose concentration was 840 mg/l with no significant difference between treatments. The post-prandial glucose profiles were characterized by a decline just after feeding, followed 3 h later by an increase. There were no significant differences between treatments in the pattern of the curves although guar gum tended to induce lower glycaemia and pectin higher glycaemia.

Plasma α-amino-nitrogen concentration rose shortly after feeding, remained high over 1 h and then dropped to a plateau in the control group. The inclusion of dietary fibre reduced the post-prandial peak of free amino-N the reduction being significant ($P<0·01$ or 0·05) with guar gum. Plasma urea concentrations rose steadily to reach peak values 4 or 5 h after feeding. Guar gum significantly reduced the post-prandial peak of plasma urea at each sampling time ($P<0·05$ or 0·01).

Plasma triglyceride concentrations increased, with a slight delay, to a plateau. The height of the plateau tended to be lower when guar gum and cellulose were added to the diet. Plasma non-esterified fatty acid concentration decreased during the first hour after feeding and then rose to a peak. The overall pattern was similar with the different diets but significantly ($P<0·05$) lower concentrations were found with guar gum. The highest concentrations in plasma cholesterol were observed before feeding with the control diet and the lowest with the guar gum-supplemented diet. Plasma cholesterol concentration before feeding varied between treatment groups and no typical pattern changes were observed after feeding. The dogs fed on the guar gum-supplemented diet showed lower concentrations of cholesterol ($P<0·05$ or 0·10).

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Systemic carnitine deficiency: an animal model produced by haemodialysis of sheep. By A. M. SNOSWELL and R. C. FISHLOCK, *Department of Animal Sciences, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia 5064* and W. B. RUNCIMAN and R. CARAPETIS, *Department of Anaesthesia and Intensive Care, The Flinders University, Bedford Park, South Australia 5042*

It is apparent that the greatest number of cases of induced carnitine deficiency in humans occurs in patients who are subjected to repeated haemodialysis for renal failure and it appeared that the availability of an animal model of this induced form of carnitine deficiency would considerably aid research in this area.

Sheep, which had previously been surgically prepared with cannulae in various blood vessels to monitor substrate and metabolite exchanges across all the major organs simultaneously (Robinson *et al.* 1984), were connected to a haemodialysis machine and dialysed at an average rate of 6·23 ml/min per kg body-weight. Dialysis for 4 h reduced the blood free-carnitine concentration to approximately 50% of the initial values ($P<0·001$) and the concentrations returned to the initial values after 18 h recovery. Carnitine balance studies showed that approximately twice the amount of carnitine lost from the blood (0·9 mmol) passed into the dialysate (1·87 mmol), indicating that carnitine was also lost from the extracellular fluid, as has been reported in humans (Böhmer *et al.* 1978). The average blood concentration (taken from eight vessels) of short-chain acylcarnitines did not vary significantly during dialysis or recovery. However, an output of short-chain acylcarnitines by the liver, as expressed by a clear difference between the hepatic venous concentration and that in the portal vein (5·5 nmol/ml, $P<0·01$) and artery (5·1 nmol/ml, $P<0·01$) occurred at 3 h recovery (Table) and also at 18 h. At 18 h an uptake of short-chain acylcarnitine by the hind-body also occurred, as evidenced by the difference between the arterial concentration and that in the blood of the inferior vena cava (3·2 nmol/ml, $P<0·01$).

Output of short-chain acylcarnitines by the liver in sheep after 3 h recovery from haemodialysis

Sheep	Blood short-chain acylcarnitines (nmol/ml) H–P	H–A	Liver blood flow rate (ml/min)	Portal blood flow rate (ml/min)	Hepatic artery blood flow rate (ml/min)
1	9·0	8·7	2150	1720	430
2	4·8	4·5	1830	1480	350
3	2·8	2·2	2900	2410	490
Mean	5·5	5·1	2330	1870	430

H, hepatic venous concentration; P, portal venous concentration; A, arterial concentration.

Liver and portal blood flows were measured by indicator dilution methods using *para*-aminohippurate infused into the mesenteric vein.

Overall, the results suggest that haemodialysis of sheep provides a useful model of systemic carnitine deficiency and suggest that treatment with acetylcarnitine or propionylcarnitine (the major components of the short-chain acylcarnitine fraction of blood) could be an efficient means of supplying carnitine replacement therapy.

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Zinc in breast milk: a fractionation study, and comparison between communities. By C. J. BATES and HARUMI TSUCHIYA, *MRC Dunn Nutrition Unit, Milton Road, Cambridge CB4 1XJ*

Many published studies have shown that the zinc content of human milk declines by a factor of around 10, between colostrum or transitional milk and the mature milk that is secreted in late lactation. Calculation has suggested that the Zn content of milk in late lactation may be insufficient for growing infants (Krebs *et al.* 1985). Zn in milk exists in several compartments, which may vary in their availability to the infant. One aim of the present study was to investigate the changing pattern of compartmentation during prolonged lactation. A second aim was to compare human milk Zn levels between a developing country (The Gambia) and a Western community (Cambridge, England), in view of current uncertainty about the factors which determine the Zn content of human milk.

Five hundred and eighty samples of milk, obtained between infant feeds, from fifty-six lactating mothers living in a rural Gambian village, covered the post-partum interval 0–24 months. Ninety-two samples from fifty-seven Cambridge mothers were obtained, mainly during the 0–12 month post-partum interval. Zn contents were measured by atomic absorption spectrometry both on unfractionated samples and on dry-ashed fractions after a centrifugal separation into the fat layer, the soluble whey fraction, and the sedimentable casein fraction.

Zinc in fractions from pooled Gambian breast milk samples (μg/ml milk)

Period post-partum (weeks)	0–7	12–18	24–30	36–38	48–56	60–68	84–87	98–103
Fat fraction	0·40	0·48	0·30	0·36	0·51	0·31	0·29	0·30
Whey fraction	2·80	1·17	1·06	1·08	0·51	0·44	0·59	0·49
Sediment	0·75	0·22	0·33	0·31	0·24	0·19	0·22	0·19

The Table shows the distribution of Zn between fractions of Gambian milks at different intervals post-partum. That in the fat fraction remained nearly constant, that in the whey fraction declined steeply, and that in the sedimentable fraction declined more moderately. The total concentration of Zn in Gambian milks was significantly ($P<0·001$) greater than that in the Cambridge milks, either by Genstat regression analysis, or by paired t test comparison of within-subject mean values at matched post-partum intervals. Neither the age nor the parity of the Gambian mothers correlated significantly with their milk Zn levels. Daily Zn intakes of Cambridge infants from breast milk alone, using milk volumes given by Prentice *et al.* (1986), were consistently below those of Gambian infants, at all stages of lactation. Cambridge infants, however, are usually weaned much sooner than Gambian infants. If Zn deficiency can occur in breast-fed infants, then the risk appears to be greater in those Cambridge infants who are breast-fed for prolonged periods, than in Gambian infants.

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Plasma methylmalonate and urocanate as indicators of defects in vitamin B_{12}-dependent metabolism in cobalt-deficient sheep. By J. PRICE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Methylmalonate (MMA), an intermediate in propionate metabolism, is elevated in plasma in the cobalt/vitamin B_{12}-deficient sheep, and Rice *et al.* (1987) concluded that MMA values >5 μmol/l are diagnostic for the deficiency. Although Gawthorne (1968) has shown that 1-carbon metabolism is also adversely affected, the degradation of histidine via urocanate being blocked at formiminoglutamate, the diagnostic potential of folate and intermediates of histidine catabolism in plasma have not been evaluated. The relative sensitivities of propionate and 1-C metabolism to vitamin B_{12} depletion have therefore been assessed using plasma MMA, 5-methyltetrahydrofolate (MeTHF) and urocanate as indicators for defects in vitamin B_{12}-dependent pathways.

One-year-old Suffolk Cross ewes (*n* 3, A, B and C) were fed on a low-Co hay (0·034 mg Co/kg dry matter) *ad lib.* for 32 weeks to induce vitamin B_{12} depletion; a parallel control group (*n* 3) was maintained in adequate vitamin B_{12} status by weekly administration of 250 μg hydroxocobalamin subcutaneously and 5 mg Co orally. A chronic indwelling catheter was established in the jugular vein. At fortnightly intervals histidine (0·05 g/kg live weight) in sterile solution was infused intravenously (i.v.) over 10 min. Plasma urocanate was determined before and at seventeen time points in the 6 h following infusion, and the area under the response peak calculated. Plasma vitamin B_{12}, MMA and MeTHF were also determined in pre-infusion samples. Propionate loading was not carried out since preliminary studies on vitamin B_{12}-deficient sheep (MMA >20 μmol/l) indicated that i.v. infusion of this substrate at levels above 0·05 g/kg live weight were poorly tolerated by the animal, while lower levels had no effect on the already elevated plasma MMA.

In control animals, plasma vitamin B_{12} concentrations were maintained above 300 pmol/l while plasma MMA and MeTHF remained consistently below 5 μmol/l and 7 nmol/l respectively, indicating vitamin B_{12} adequacy. Within this group the overall mean for plasma urocanate before histidine loading was 0·24 (SD 0·23) μmol/l and that for the area under the urocanate response peak was 218 (SD 154) units following loading.

Plasma vitamin B_{12} in the Co-depleted group declined from a mean initial value of 300 (SD 18) pmol/l to deficiency levels (<185 pmol/l) within 8 weeks. Increases in plasma MMA above 5 μmol/l were detectable in individual sheep at 6(A), 9(B) and 16(C) weeks, but plasma MeTHF remained below 7 nmol/l and pre-infusion urocanate levels showed no change during depletion with the overall mean, 0·23 (SD 0·20) μmol/l, not differing significantly from that of the control group. Plasma urocanate response to histidine infusion did not increase above control levels until weeks 16(A), 22(B) and 32(C), at which times response peak areas were 2257, 2163 and 1301 units respectively.

These results indicate that propionate metabolism is affected earlier in vitamin B_{12} deficiency than is 1-C metabolism and that MMA is the most sensitive indicator of a functional deficiency of the vitamin in non-productive sheep.

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Regulation of long bone growth in vitro: effects of insulin-like growth factor-1 and growth hormone. By BEN A. A. SCHEVEN and NICOLA J. HAMILTON, *Bone Growth and Metabolism Unit, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB* (Introduced by P. TRAYHURN)

An in vitro model system was developed to study longitudinal growth of rat long bones. Long bones were dissected from fetal or neonatal rats and cultured in a chemically-defined, serum-free medium supplemented with bovine serum albumin (BSA). During culture the longitudinal growth of the long bones was followed by measuring the lengths of the bones at different time points using an ocular graticule mounted in an inverted or stereo microscope. Fetal long bones entirely composed of growth plate chrondrocytes show a steady rate of growth for at least 7 d in serum-free media. [^{3}H]thymidine incorporation experiments confirmed that the enlargement of the bones was due to cellular proliferation. This indicates that the fetal bone explant itself produces autocrine/paracrine growth factors stimulating chondrocyte proliferation. Addition of recombinant insulin-like growth factor (IGF, 50 ng/ml) to the medium resulted in a direct stimulation (+40%) of the long bone growth. Interestingly, recombinant human growth hormone (GH, 50 ng/ml) was also able to stimulate the longitudinal growth, although this was accomplished after a lag time of more than 2 d. Studies with a monoclonal antibody to IGF-1 indicated that the GH-stimulated growth was caused by local induction of IGF-1 production. The antibody could not inhibit the growth of the bones in control medium, suggesting that other local factors were important for fetal growth.

Unlike the fetal bones, long bones from 2-d-old neonatal rats were arrested in their growth after 1–2 d in vitro. This implies the possibility that systemic factors are becoming increasingly more important for the regulation of long bone growth after birth. The neonatal bones responded to IGF-1 and GH in a similar fashion to the fetal bones.

In conclusion, this study shows that (1) endogenous growth factors play an important part in the regulation of long bone growth and (2) GH acts directly on growth plate chondrocytes and induces them to produce IGF.

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Investigation of the effects of insulin-like growth factor-1 and age on cartilage growth using *in situ* biochemical techniques. By COLIN FARQUHARSON and NIGEL LOVERIDGE, *Bone Growth and Metabolism Unit, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB* (Introduced by J. ARTHUR)

It is well accepted that there are several growth factors involved in the regulation of skeletal growth. Through interaction with cell surface receptors they are presumed to have a specific role in the growth of the skeleton. Previous studies have used isolated cells to assess changes in the biochemistry and proliferation rate of specific cells in response to growth mediators. *In situ* methods, however, allow the study of particular bone cells in their natural environment. Current methods for the investigation of cell proliferation in histological preparations are based on the autoradiographic analysis of [^{3}H]thymidine uptake. Recently, the bromodeoxyuridine (BrdUrd) antibody staining technique has been shown to offer superior resolution and speed in the determination of the labelling index of proliferating cells (Vogel *et al.* 1986). In the present study we have used the BrdUrd technique in conjunction with organ culture to assess the effect of age and insulin-like growth factor-1 (IGF-1) on growth plate chondrocyte proliferation.

The metatarsals were removed from rats of 1–10 weeks of age and were exposed to 50 μM-5-bromodeoxyuridine for 1 h before being chilled and sectioned. The nuclei were stained for the presence of deoxyuridine with a specific monoclonal antibody. Unstained nuclei were visualized by propidium iodide. The labelling index was the percentage of cells that were positive for BrdUrd. Serial sections were reacted for glucose 6-phosphate dehydrogenase (G6PD) determination as it has been suggested that the activity of this enzyme is related to cell proliferation (Coulton, 1977). The labelling index decreased from 10·7 (SE 1·9)% in 1-week-old rats to 5·3 (SE 1·9)% at 10 weeks and was significantly correlated with age ($P<0·001$). This is in contrast to G6PD activity within the chondrocytes where no significant changes were noted.

Metatarsals from 4-week-old rats were incubated for 6 h in serum-free medium supplemented with 10 ng IGF-1/ml and, for the last 1 h, with BrdUrd. The labelling index was 8·2 (SE 1·7)%, significantly higher ($P<0·01$) than that in unsupplemented control bones (5·8 (SE 1·9)%). BrdUrd uptake in the growth plate was generally confined to the chondrocytes of the proliferating zone. Limited staining was seen in the resting chondrocytes and none at all in the hypertrophic and degenerative zones.

In conclusion, the results demonstrate the usefulness of the BrdUrd technique as applied to bone and indicate that chondrocyte proliferation is enhanced by IGF-1 but decreased with age. They also cast doubt on the relationship between G6PD activity and cell proliferation.

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Effects of growth hormone and nutrient interactions on ovine adipose tissue metabolism.

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Long-term treatment of weaned lambs with bovine growth hormone (bGH) can decrease fat accretion in both visceral and subcutaneous tissue, reducing its contribution to total body-weight. The mechanism by which bGH limits fat deposition could be via inhibition of synthesis or stimulation of breakdown, or a combination of both; this may be influenced by energy intake.

Nine-week-old lambs (mean initial weight 21·3 kg) were randomly allocated into four groups (six per group) and were fed on a concentrate diet (200 g crude protein/kg, Pell *et al.* 1989) either *ad lib.* (A, average intake 50 g/kg live weight (LW) per d) or restricted (R) to 30 g/kg LW per d. Within each dietary group lambs were injected subcutaneously with either saline (–, 9 g sodium chloride/l) or recombinant bGH (+, 0·1 mg/kg LW per d) until slaughter at 19 weeks. Samples of visceral (V) and subcutaneous (S) tissue were removed rapidly at slaughter and small pieces were used to measure [1–^{14}C]acetate incorporation into triacylglycerol (lipogenesis) and adrenaline-stimulated glycerol release (lipolysis). Adipocyte size was determined on osmium tetroxide fixed tissue. Differences between groups were assessed by analysis of variance using a split plot design.

Treatment		R−	R+	A−	A+	SE	Main effects: Level	Main effects: bGH
Fraction of fat in carcass		0·222	0·185	0·299	0·254	0·017	***	*
Cell volume (pl)	V	475	503	780	688	71·0	**	NS
	S	436	462	778	651	64·9	***	NS
Lipogenesis†	V	5·8	4·8	8·3	7·9	0·99	**	NS
	S	5·7	2·9	11·2	10·7	2·37	**	NS
% Insulin stimulation of lipogenesis	V	+44	+58	+12	+18	11	**	NS
	S	+25	+64	+14	+19	11	*	NS
Lipolysis†	V	2·89	1·86	2·41	2·44	0·42	NS	NS
	S	3·06	1·73	2·11	2·02	0·47	NS	NS

NS, not significant. * $P<0·05$, ** $P<0·01$, *** $P<0·001$.

† Activities are expressed as μmol substrate converted or formed per 2 h per 10^6 cells.

Animals on the restricted diet had significantly less carcass fat, smaller adipocyte volumes and reduced lipogenic rates but greater insulin stimulation of lipogenesis. The fraction of fat in the carcass was significantly reduced in bGH-treated lambs but there were no significant differences ($P>0·05$) in lipogenic or lipolytic rates overall. However, with R+, both tissues showed a marked decline in lipolysis while a decline in lipogenesis was observed in the subcutaneous tissue. These results differ in direction and magnitude from those reported by Pell *et al.* (1988) where lambs were injected every other day with 10 mg pituitary GH for 16 d. This highlights the need to take account of the protocol of GH treatment together with the physiological and nutritional state of the animal when interpreting the response to the hormone.

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Influence of intravenous glucose infusion on basal and growth hormone-stimulated plasma concentrations of insulin-like growth factor 1 in lambs. By G. V. KRIEL, M. J. BRYANT and M. A. LOMAX, *School of Animal and Microbial Sciences, University of Reading, Whiteknights, PO Box 228, Reading RG6 2AJ*

Plasma concentrations of total insulin-like growth factor 1 (IGF-1) and the IGF-1 secretory response to injected growth hormone (GH) are higher in steers fed on a high level of feed intake compared with steers fed below maintenance (Breier *et al.* 1988). The objective of the present study was to determine whether this interaction between nutrition and IGF-1 secretion could be mediated by changes in plasma glucose and insulin concentrations.

Six wether lambs, average live weight 35·0 (SE 0·6) kg, were fed once daily on a diet comprising 640 g concentrate/d (Lamlac, start to finish, Volac Ltd, Herts) and 80 g chopped straw/d at an estimated maintenance energy intake of 6·7 MJ/d. In a cross-over design, lambs were infused with either saline (9 g sodium chloride/l) (0·25 ml/min) or glucose (0·015 mmol/kg per min; average 2·1 MJ/d) via a jugular vein catheter for 6 d. On the 4th day of infusion, blood samples were taken at hourly intervals via a second jugular vein catheter, during the period 09.00 to 18.00 hours. Ovine GH (0·1 mg/kg) was then injected into the jugular vein and after 12 h samples taken at approximately 2-h intervals for the next 24 h. Samples were analysed for plasma concentrations of total IGF-1, insulin and glucose. The results are shown in the Table.

	Saline			Glucose		
Plasma concentration	Mean	SEM	*n*	Mean	SEM	*n*
Glucose (mM)	4·67	0·24	6	6·19***	0·17	4
Insulin (ng/ml)	1·03	0·31	6	2·05*	0·19	6
Basal IGF-1 (ng/ml)	179	17	6	275***	10	6
Peak IGF-1 (ng/ml)	364	7	4	482**	30	4
Area under IGF-1 peak (ng/ml per h)	98	3	4	110	6·8	4

Significantly different from saline infusion (Student's *t* test): * $P<0·05$, ** $P<0·01$, *** $P<0·001$.

Glucose infusion significantly ($P<0·05$) increased the plasma concentrations of glucose, insulin and IGF-1. The plasma IGF-1 peak response to injected GH was significantly increased ($P<0·01$) but there was no difference between treatments in the time from GH injection to the IGF-1 peak (saline 26·0 (SE 2·2), glucose 27·4 (SE 1·1) h).

It is concluded that glucose infusion into maintenance-fed lambs stimulates basal plasma IGF-1 concentrations but does not alter the plasma IGF-1 response to injected growth hormone.

Breier, B. H., Gluckman, P. D. & Bass, J. J. (1988). *Journal of Endocrinology* **118,** 243–250.

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Interactions between protein supply and the effect of growth hormone administration on plasma insulin-like growth factor 1 concentrations in infusion-maintained lambs. By J. C. MACRAE, J. E. INKSTER, L. A. BRUCE, F. D. DEB. HOVELL and T. ATKINSON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Exogenous growth hormone (GH) systematically enhances the productive performance of lactating animals, probably via the action of insulin-like growth factor 1 (IGF-1) (Hart, 1988). In contrast, GH administration has not universally promoted lean tissue accretion in experiments with growing animals, possibly because the efficacy of GH administration may relate to protein status.

Intragastric infusion of all nutrients (Ørskov *et al.* 1979) provides a means of regulating precisely the amounts of amino-nitrogen available to the animal. This technique has been used and the changes in plasma IGF-1 concentrations measured in response to exogenous GH when sheep were infused with either 600 (LP) or 1200 (HP) mg casein N/kg body-weight$^{0.75}$ per d (with energy held constant at 800 kJ/kg body-weight$^{0.75}$ per d). Six sheep underwent a 40 d infusion consisting of 18 d LP, 4 d adjustment, 18 d HP, or vice versa. Over days 6–12 of each 18 d period each animal received a continuous intravenous infusion of recombinant bovine GH (4·5 mg/d). Plasma IGF-1 concentrations were monitored daily in three of the animals. In the other three they were monitored on the last day of each 6 d sub-period. Fig. 1 shows the daily changes in plasma IGF-1 concentration in the former animals; the data for sheep no. 2 have been transposed because the HP infusion was from days 1–18 and the LP infusion from days 22–40 in that animal.

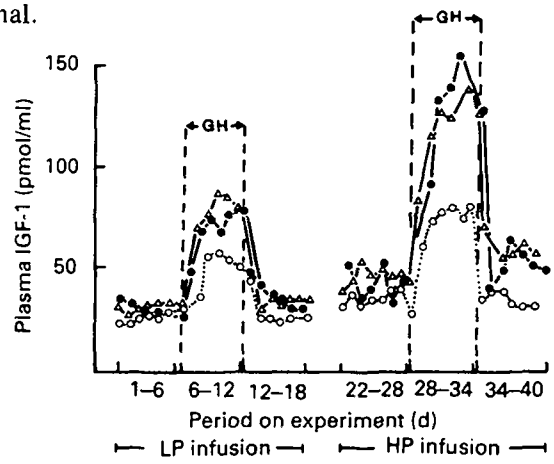

Fig. 1. Plasma IGF-1 concentrations in three individual lambs (lamb 1 ○ - - - ○, lamb 2 ●——●, lamb 3 △——△) given a low protein (LP) or high protein (HP) intragastric infusion for 18 d and an intravenous infusion of GH over days 6–12 of each 18 d period.

Analyses of the combined data for all six sheep indicate that control plasma IGF-1 concentrations were greater (13 (SE 2·2) pmol/ml; $P<0·01$) and responses to GH infusion larger (25 (SE 7·9) pmol/ml; $P<0·05$) when the sheep were receiving the HP infusion. This difference in response of plasma IGF-1 concentration to GH administration reflected the corresponding short-term responses in N retention, which were greater ($P<0·05$) on the HP ration.

The authors thank Dr Ian Hart of the American Cyanamid Co., for the gift of recombinant GH.

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Nutritional control of circulating insulin-like growth factor-1 and growth hormone concentrations in growing pigs. By J. M. FLETCHER, P. T. QUINLAN and A. D. HEATH, *Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ*

The poor growth of animals during undernutrition is associated with a low plasma concentration of insulin-like growth factor-1 (IGF-1) and frequently with a high concentration of growth hormone (GH) (Underwood *et al.* 1986). The hormonal control of growth and nutrient partitioning in animals receiving a diet adequate to support rapid growth is, however, not well understood. We have carried out two studies to determine whether circulating IGF-1 concentration is associated with growth of well-nourished, rapidly growing pigs.

Male pigs were fed on isoenergetic diets at three different protein concentrations (150, 200 and 250 g/kg diet). The amount of energy given daily was estimated to provide approximately 2·5 times the maintenance requirement. Nitrogen and energy balances were measured using a randomized block design over three 5 d periods. Approximate starting weights were 30, 35 and 45 kg. In order to avoid meal-induced fluctuations in metabolites and hormones, pigs were given a twelfth of their daily intake every 2 h. Blood samples from the ascending vena cava were taken half-hourly for one 24 h period during each balance. Blood samples were taken simultaneously from the vena cava and hepatic portal vein at 6-h intervals on the sampling day. Blood samples were analysed for insulin, GH, IGF-1, cortisol, glucose and amino acids.

N retention was poorly associated with dietary protein, but showed a marked effect of age. IGF-1 concentrations did not significantly differ throughout a 24 h period. There was no significant difference in IGF-1 concentration between portal and vena cava blood for any balance period. The N retention of individual pigs (g N retained/d) was significantly correlated with the mean of the 24 h IGF-1 concentration (r 0·76, $P<0·001$, n 12). Insulin, cortisol and GH concentrations were not correlated with either N retention or mean IGF-1 concentrations. The efficiency of N retention of individual pigs (g N retained/g N intake) was, however, significantly correlated with the mean of the 24 h GH concentration (r 0·50, $P<0·05$, n 12).

In a second study male pigs (25–35 kg) were fed on isoenergetic diets at two different protein concentrations (150 and 250 g/kg diet). N and energy balances were determined over two 5 d periods, as described above. Animals were fed twice daily, and blood samples from a carotid catheter obtained at hourly intervals over one 24 h period during each balance. N retention was significantly greater ($P<0·01$, n 5) and plasma IGF-1 concentrations were significantly higher ($P<0·001$) for pigs fed on the diet containing 150 g protein/kg.

It is concluded that circulating IGF-1 may play an important role in the control of lean deposition in young, rapidly growing pigs. In pigs fed on diets supporting high growth rates, GH does not appear to be an important anabolic stimulus, but it may influence the efficiency of N utilization.

IGF-1 assays in the first study were performed by Drs A. Holder and D. Morrell of The Institute for Child Health, London.

Underwood, L. E., Clemmons, D. R., Maes, M., D'Ercole, A. J. & Ketelslegers, J.-M. (1986). *Hormone Research* **24**, 166–176.

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The influence of dietary composition on the growth of the domestic cat. By K. E. EARLE, H. S. MUNDAY and P. M. SMITH, *Waltham Centre for Pet Nutrition, Freeby Lane, Waltham-on-the-Wolds, Melton Mowbray, Leicestershire LE14 4RT*

The domestic cat has evolved as an obligate carnivore and has special dietary requirements which distinguish it from other carnivores. A number of the nutrients essential to the cat can only be derived from animal tissues (e.g. taurine, vitamin A) and their metabolism demands a high basal protein requirement (14% of the diet).

The aim of the present study was to assess the effect of dietary composition and total energy intake on kitten growth.

Kittens were weaned at 8 weeks and divided into four groups of twelve, paired for sex and body-weight. Earlier studies at the Waltham Centre for Pet Nutrition (Loveridge, 1987) showed a significant difference in body-weight between male and female kittens as early as 6 weeks of age. Where possible littermates were given different diets, with food offered *ad lib*. Body-weight was measured weekly and food intake daily for 10 weeks. The compositions of the four diets are shown in the Table.

	Diets			
% Energy as:	A	B	C	D
Protein	62	48	46	46
Fat	33	36	46	49
Nitrogen-free extract	5	16	8	5
Predicted metabolizable energy* (kJ/kg)	2850	2800	3400	2470

* Kendall *et al.* (1985).

Diets A and B were of similar energy content but different composition, whilst diets C and D were of similar composition but different energy content.

Mean food intake, energy intake and body-weight gain were not significantly different between groups A and B. Maximum energy intake for groups A and B was recorded at week 10 (963 kJ/kg body-weight per d) and declined linearly to 670 kJ/kg body-weight per d by week 18. Group D had significantly higher food intakes/d than group C. However, daily energy intakes for groups C and D were the same and not significantly different from groups A and B. Group A had a higher protein energy intake than group B but showed no difference in body-weight gain; groups C and D had similar protein energy intakes and yet the body-weight gain of group D was significantly lower than for group C.

The rate of growth of group D was significantly lower than that of groups A, B and C up to 13 weeks (time of highest energy demand); from weeks 14 to 18 the growth rate increases were similar for all four groups. The kittens in group D had a mean body-weight of 1735 g at 18 weeks which was significantly lower than groups A, B and C (2066, 2006 and 2087 g respectively).

The growth of the kittens was dependent on the total energy intake, which they regulated by increasing food intake on the lowest energy diet. Changing the composition of the diet to supply more protein had no effect on growth rate even though kittens are known to have a high demand for this nutrient.

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