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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Sixty-first Meeting of the Nutrition Society was held in the Barnes Lecture Theatre of the Royal Society of Medicine, London, on Thursday, 7 May, 1981, *when the following papers were read:*

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A longitudinal study on the effect of riboflavin status on aspects of iron storage in the liver of growing rats. By D. A. ADELEKAN and D. I. THURNHAM, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

Riboflavin deficiency in the rat has been associated with several haematological changes including a reduction in erythropoiesis (Hassan & Thurnham, 1977), a fall in plasma iron turnover (Jamdar *et al.* 1968) and decrease in stored Fe in the liver (Sirivech *et al.* 1977). Measurements of Fe storage in the liver at different stages of riboflavin deficiency are reported here.

Male, weanling, albino rats (Wistar, approx 50 g) were divided into four groups: control (C), pair-fed (PF), weight-matched (WM) and riboflavin-deficient (RD). The diet of C, PF and WM rats were supplemented with 44 mg riboflavin/kg diet. Determinations of riboflavin status (Hassan & Thurnham, 1977), total non-haem Fe (Foy *et al.* 1967) and ferritin iron (Drysdale & Ramsay, 1965) were made at the start of the experiment and subsequently at 14, 21, 28, 35 and 49 d. Riboflavin status was expressed as an activation coefficient (AC).

Percentage increase in concentration (μg/g liver) of stored Fe over 49 d

	Control	Pair-fed	Weight-matched	Riboflavin-deficient
Total non-haem	194	180	185	35
Ferritin Fe	115	115	154	54

The AC (mean ± SD) in the RD rats increased from 1·06±0·06 to 2·70±0·27 at 49 d; there was no change in the C, PF and WM rats. The Table shows that C, PF and WM rats stored increasing amounts of Fe in their livers during the course of the experiment. By contrast, rats in the riboflavin-deficient group stored very little Fe; the amount of Fe in their livers falling behind that of control values from the 21st d of the experiment. The results suggest that Fe storage is impaired after 3 weeks on a riboflavin-deficient diet but this may be secondary to a blockage in the mobilization of Fe from the gut as proposed by Sirivech *et al.* (1977).

D.A.A. is supported by the University of Ife, Nigeria and D.I.T. by the Department of Health and Social Security.

Drysdale, J. W. & Ramsay, W. N. M. (1965). *Biochem. J.* **95**, 282.
Foy, A. L., Williams, H. L., Cortell, S. & Conrad, M. E. (1967). *Analyt. Biochem.* **18**, 559.
Hassan, F. M. & Thurnham, D. I. (1977). *Int. Z. Vitaminforsch.* **47**, 349.
Jamdar, S. C., Udupa, K. B. & Chatterji, A. (1968). *Vitaminol.* **14**, 219.
Sirivech, S., Driskell, J. & Frieden, E. (1977). *J. Nutr.* **107**, 739.

0029–6651/81/4003–361A $00.35

The protein requirement of adult cats. By I. H. BURGER, S. E. BLAZA and P. T. KENDALL, *Animal Studies Centre*, Waltham-on-the-Wolds, Melton Mowbray, Leics LE14 4RT*

The cat has traditionally been regarded as having a higher protein requirement than other mammals. Greaves & Scott (1960) concluded that 21% protein (dry matter) was necessary to maintain adult resting cats in nitrogen equilibrium while several reports (Dickinson & Scott, 1956; Miller & Allison, 1958; Jansen *et al.* 1975) indicate that protein requirements for growth are met only by diets containing more than 30% protein. However, these results were complicated by a lack of information on the cat's amino acid needs. Recent work in the USA has more closely defined the amino acid requirements of growing kittens by the use of purified amino acid diets; it was reported last year (Anderson *et al.* 1980) that if all essential amino acids are present at the correct concentrations, maximum growth can be achieved by only 16% protein in such a diet.

Using soya protein-based semi-purified diets (ME 4·5 kcal/g), we are investigating the protein requirement of adult cats, in the light of this new information. The essential amine acid profile of each diet is adjusted to meet the values obtained by Anderson *et al.* In the first experiment a group of nineteen cats was fed on 17, 13 or 10% dietary protein for 4 weeks in a modified Latin Square design. Regression analysis of N balance results indicated N equilibrium at an intake of (mean±SEM) 280±67·4 mg N/kg body-weight per d, equivalent to approximately 12% dietary protein (10% protein energy). In a second experiment we are feeding this protein level over a longer period to measure N balance and observe the effects of the diet on the general health of the cats. Results of the first N balance determination show an over-all (positive) balance of (mean±SD) 19·8± 27·7 mg N/kg per d with fourteen out of nineteen cats in positive balance. N intake was 266±36·2 mg/kg per d; urine and faecal N outputs were 199±34·5 and 47·2± 16·4 mg/kg per d respectively. These values, together with the good health and condition of the cats after about four months on the diet, suggest that a dietary protein level of about 12% is sufficient to maintain adult cats in N equilibrium.

Anderson, P. A., Baker, D. H., Sherry, P. A. & Corbin, J. E. (1980). *Am. J. Vet. Res.* **41,** 1646.
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Jansen, G. R., Deuth, M. A., Ward, G. M. & Johnson, D. E. (1975). *Nutr. Rep. Int.* **11,** 525.
Miller, S. A. & Allison, J. B. (1958). *J. Nutr.* **64,** 493.

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Nitrogen solubility and protein degradability of commercially and laboratory prepared rapeseed and soya-bean meals. By KATHRYN A. LAYCOCK and E. L. MILLER, *Department of Applied Biology, University of Cambridge, Pembroke St., Cambridge CB2 3DX*

Nitrogen solubility of different classes of feedstuffs does not correlate with extent of protein degradation in the rumen. However, within one class of feedstuff N solubility may be an indicator of variability in processing conditions and possibly of changes in susceptibility of the protein to degradation by rumen micro-organisms.

N solubility of fourteen commercial rapeseed meals, determined with 0·15 M-sodium chloride (Crawford *et al.* 1978) was variable (mean proportion (±SD) 0·35±0·125) indicating possible variation in heat treatment during processing.

Whole rapeseed and soya beans were ground, petroleum ether extracted (B.P. 40–60°) sealed into glass vials and heated at 111·6, 127·0 and 143·7° in an oil bath for 15, 30, 45 or 60 min. N solubility decreased curvilinearly with time and linearly with increase in temperature. N solubility of soya was reduced to a greater extent than that of rapeseed.

N solubility and 'in sacco' degradability (Mehrez & Ørskov, 1977), determined on further samples heated at 127·0 and 161·8° for 15 min are shown in the Table. Degradability (dg) was calculated from;

$dg=a+(1-a)\frac{K_d}{K_r+K_d}$ (Miller, 1980), assuming a value of 0·05/h for K_r.

	Rapeseed			Soya			
	Unheated	127·0	161·8	Unheated	127·0	161·8	SEM
N solubility	0·56	0·37	0·28	0·50	0·18	0·12	0·011
a	0·47	0·26	0·24	0·27	0·12	0·15	0·028
K_d	0·17	0·16	0·11	0·22	0·15	0·11	0·018
dg	0·89	0·83	0·77	0·86	0·78	0·74	0·021

Heating reduced N solubility, the proportion of N rapidly leaving the polyester bag (a) and the rate of degradation of the insoluble fraction (K_d). Regression of degradability on N solubility in 0·15 M NaCl gave dg=0·33 (N solubility)+0·70 (r 0·97; RSD 0·015; $P<0·01$) with no difference in the relationship for rape or soya samples.

Crawford, R. J., Hoover, C. J., Sniffer, C. J. & Cooke, B. A. (1978). *J. Anim. Sci.* **46**, 1768.
Mehrez, A. Z. & Ørskov, E. R. (1977). *J. agric. Sci., Camb.* **88**, 645.
Miller, E. L. (1980). Vicia faba. *Feeding Value, Processing and Viruses*, p. 17 [D. A. Bond, editor]. Brussels: Martinus Nijhoff.

0029–6651/81/4003–361A $00.35

A method for measuring short-term changes in duodenal outflow rate.

By S. JAMES, J. B. ROWE and A. W. J. BROOME, *Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG*

Methods for measuring rumen volume, turnover rate and the rate of fluid flow to the small intestine are well established using either a continuous infusion or a single injection of marker (e.g. Cr-EDTA) into the rumen. The periods of time over which measurements are necessary to satisfy the requirements of marker dilution techniques are impractically long for the purpose of studying short-term effects of drugs on rumen motility and fluid turnover rate. The method reported here was based on the theory that a marker, infused into the duodenum, would mix rapidly with digesta and therefore provide a sensitive method of estimating changes in the flow of digesta from the forestomachs over relatively short time periods.

A mature sheep, weighing 45 kg, was prepared with a permanent cannula in the rumen and duodenum ('T' cannula approximately 0·5 m posterior to the pyloric sphincter) and a silicon rubber infusion line fixed into the duodenum near the pylorus. A series of experiments was conducted to investigate the possibility of altering the strength and frequency of rumen contractions by pharmacological means and to measure the effect of these changes on fluid flow from the forestomachs. Reticulo ruminal motility was measured using a pre-pressurized balloon placed in the reticulum. The rate of fluid flow to the small intestine was measured relative to Cr-[^{14}C]EDTA in samples of duodenal digesta. Cr-[^{14}C]EDTA was infused continuously during experimental periods through the duodenal infusion line (0·15 ml/min). Samples of duodenal digesta were taken sequentially, each sample being collected over a period of 5 min. Feeding was by a continuous belt feeder which was turned off during measurement periods.

The results of an experiment, in which 20 mg apomorphine (Sigma) followed 30 min later by 25 mg metaclopramide (Beechams, 'Maxolon') were administered intravenously, are given in the Table. Apomorphine causes gastric stasis in monogastrics via its action at the chemoreceptor trigger zone and metoclopramide reverses this through an anti-dopaminergic effect.

	Pre-injection			Apomorphine*			Metoclopramide*		
	n	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE
Flow rate (ml/min)	7	14·1	0·31	6	1·2	0·15	4	14·8	2·58
Amplitude (mV)	7	2·6	0·16	6	<0·01		4	3·8	0·41
Frequency (contractions/min)	7	1·7	0·08	6	0·2	0·18	4	2·8	0·13

*Plateau values reached 5 min after injection of the drug.

It was concluded from these studies that (1) the method provided reproducible estimates of duodenal flow rate sensitive to short-term changes in forestomach motility and (2) although an increase in duodenal fluid flow rate was observed with increased amplitude of rumen contractions it was not possible to maintain this response using pharmacological stimulation of the nervous system.

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The relationship between body-weight and survival in institutionalized elderly subjects. By C. HUNT, *Department of Catering Studies, Huddersfield Polytechnic*

Complete weighed dietary intakes were performed for 2 d each on fifty elderly subjects (twenty-eight female, twenty-two male) in five welfare homes for the elderly in a town in the North of England.

Intakes of energy and nutrients were estimated using computerized food tables. Height and weight and an assessment of state of health was also recorded for each subject. The study was carried out in 1975, and repeated in 1978 on the twenty survivors from 1975 plus thirty 'new' subjects.

There was no significant difference in mean energy consumption in 1975 between subjects who survived from 1975 to 1978 ('survivors') (mean ± SE, 6·60±0·40 MJ/d) and those who did not ('non-survivors') (6·93±0·36 MJ/d). However, there was a striking difference in the relationship between energy intake and body fatness (as measured by the percentage difference from 'ideal' body-weight); that is, in the survivors there was a strong and highly significant positive correlation between degree of fatness and energy intake, whereas in the non-survivors there was no such correlation.

Correlation between energy intake (2 d average) and difference from 'ideal' body-weight (%)

	r	*n*	*P*
Survivors 1975	0·800	19	<0·001
Non-survivors 1975	−0·162	30	>0·1
Survivors 1978	0·614	19	<0·01
'New' subjects 1978	−0·062	27	>0·1
Males 1975	0·322	17	>0·1
Females 1975	0·286	32	>0·1
Males + females 1975	0·196	49	>0·1
Males + females 1978	0·210	46	>0·1

There appears to be no obvious explanation of this difference between the two groups since it was not due to differences in sex, mean age, energy intake or degree of overweight. Survivors were relatively healthy in 1975 and most of them maintained this state of health through to 1978.

The results suggest the possibility that amongst relatively healthy old people fatter individuals eat more and thinner ones eat less, a finding which contrasts with many studies on younger people. This relationship seems to be altered by ill-health or in people whose survival prospects are poor.

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The effects of dietary protein–energy supplementation in pregnant Asian mothers in Birmingham. By O. A. C. VIEGAS, PHYLLIS EATON, JEAN KING, P. H. SCOTT, PAMELA WHARTON and B. A. WHARTON, *Sorrento Maternity Hospital, Birmingham B13*

At this hospital Asian mothers whose babies had grown poorly in utero had anthropometric and biochemical evidence of a poorer nutritional status at the end of the second trimester than Asian mothers having a well grown baby (Bissenden *et al.* 1980). We have, therefore, tested the hypothesis that dietary protein–energy supplementation in such mothers would improve intrauterine growth.

One of three supplements was given to Asian mothers during the 2nd and 3rd trimesters (n 114) or during the 3rd trimester only (n 116). The supplements were (a) vitamins only (vitamin C 30 mg/d for those supplemented in the 2nd and 3rd trimesters; a multivitamin sachet containing vitamins A, B, C and D for those supplemented in the 3rd trimester only). (b) Energy plus vitamins (10 000–30 000 kcal/trimester all from carbohydrate; vitamins as in (a)). (c) Protein–energy plus vitamins (energy and vitamins as in (b) but 5–10% of energy from milk protein).

Mothers receiving the protein–energy supplement during the second trimester had by 28 weeks put on more weight and more fat than the vitamin group (weight increment 0·47 *v.* 0·36 kg/week $P<0·05$; triceps skinfold increment 0·19 *v.* 0·02 mm/week $P<0·05$). Similar differences occurred during the third trimester but did not reach statistical significance.

Over all, the protein–energy supplementation (when compared to vitamins alone) was not associated with an increased crude birth weight (3·026 kg *v.* 3·028 kg *v.* 3·075 kg) nor with a significant increase in birth weight centile (median centiles 42 *v.* 34 *v.* 32). However, in the seventy-six mothers with uncomplicated pregnancies who from the evidence of our previous work (Bissenden *et al.* 1980) had put on fat inadequately during the second trimester (i.e. triceps increment $\leqslant$0·02 mm/week) the protein–energy supplement, compared to vitamins alone, was associated with an increased crude birth weight (3·182 kg *v.* 2·977 kg; $P\simeq0·07$) and a higher weight centile for gestational age (median centile 48 *v.* 25; $P=0·01$); protein–energy supplementation did not lead to improved intrauterine growth in those mothers who had put on fat adequately.

This differential effect of supplementation depending on the mothers nutritional status during the second trimester may explain apparently conflicting results of other studies where some have shown a substantial effect of supplementation (Lechtig *et al.* 1975) and others only a small one (Rush *et al.* 1980). This effect of intervention is further evidence that 'poor nutriture' contributes to poor intrauterine growth in selected mothers even in developed countries.

Bissenden, J. G., Scott, P. H. & Wharton, B. A. (1980). *Proc. Nutr. Soc.* **38,** 103A.
Lechtig, A., Delgado, H., Lasky, R., Yarborough, C., Klein, R. G., Habicht, J. P. & Behar, M. (1975). *Am. J. Dis. Childh.* **129,** 553.
Rush, D., Stein, Z. & Susser, M. (1980). *Paediatrics, Springfield* **65,** 683.

0029–6651/81/4003–361A $00.35

Height, weight, 'fatness' and body-build of a large sample of British adults. By F. C. McKay, S. Grant and J. V. G. A. Durnin, *Institute of Physiology, University of Glasgow*

The main object of this study is to set up new standards for assessing the desirable weight of British adults and in particular for men and women in the Armed Forces. These standards will encompass a variety of builds, and will include the range of ages from 16–50 years. Data will be collected from approximately 12 000 males and females, taken from a broad selection of jobs and locations, but biased to parallel the over-all population distribution in Britain, as far as possible.

The measurements taken are height, weight, ulnar, tibial, biacromial and biiliac diameters, upper arm, calf, thigh and buttocks circumferences, as described by Weiner & Lourie, and biceps, triceps, subscapular and suprailiac skinfolds as described by Durnin & Rahaman (1967). Using the equations of Durnin & Womersley (1974) percentage fat is calculated for each subject, from the sum of skinfolds. All measurements are taken with the subjects in their underwear.

Additional social information on personal background, exercise frequency and smoking is also collected from each subject.

Only three field workers have been involved in collecting these measurements and the reproducibility of measurements between field workers is checked constantly, thus ensuring a high degree of standardization.

At present 3440 males and 370 females have been examined, all from the armed forces, but as this is an ongoing project, by the summer it is hoped that these numbers will have reached 5500 and 1000 respectively. Numbers in each male age group are shown in the Table.

Age (years)	<17	17–19	20–24	25–29	30–34	35–39	40–45	>45
n	115	881	990	457	425	310	155	107

Results, so far, on males have shown the average height to be 173–2 cm (SD±6·7), weight 69·8 kg (SD±10·6) and fat content as 17·0% (SD±5·3). These height and weight figures are similar to those of Montegriffo (1968) who had over-all averages of 175 cm and 72·6 kg (with clothing) respectively.

The percentage fat figure is, however, higher than any 'ideal' value quoted, which is surprising considering that 58% of the sample was under the age of 24 years and might be expected to be leading moderately active lives.

This work has been carried out with the support of the Chief Scientist (Army), Ministry of Defence.

Durnin, J. V. G. A. & Rahaman, M. M. (1967). *Br. J. Nutr.* **21**, 681.
Durnin, J. V. G. A. & Womersley, J. (1974). *Br. J. Nutr.* **32**, 77.
Montegriffo, V. M. E. (1968). *Ann. Hum. Genet., Lond.* **31**, 389.

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A comparison of the energy and nutrient intakes of 305 Glasgow infants with current recommendations for the UK. By F. M. MCKILLOP and J. V. G. A. DURNIN, *Institute of Physiology, University of Glasgow*

This investigation was an attempt to examine relationships between nutritional intake and growth, 'fatness' and socio-economic status of infants living in an economically deprived area in the UK.

The study consisted of a 5 d weighed food intake and anthropometric examination of 305 Glasgow infants of varying socio-economic backgrounds, aged between 3 months and 2 years. The dietary intakes of energy, protein calcium and iron were compared with the current recommendations for the UK (DHSS, 1979).

65% of infants aged 3–12 months and 78% of infants between 12–24 months had mean energy intakes which fell below the RDA.

Age (months)		Mean daily energy intake (MJ)	(RDA)	Energy/kg per d (MJ)	(RDA)
3–12	♂	3·44	(3·60)	0·39	(0·42)
	♀	3·09	(3·33)	0·40	(0·42)
12–24	♂	4·84	(5·44)	0·42	(0·46)
	♀	4·67	(5·02)	0·43	(0·45)

In the 3–6-month-old group, 35% had mean protein intakes below the RDA of 2·5 g/kg per d (mean 2·75 g/kg per d). In the 6–12-month-old group (RDA 2·6 g/kg per d) and the 12–24 month-old group (RDA 2·8 g/kg per d), 15 and 22% respectively failed to meet the recommended levels.

For Ca, nearly 70% of 3–6-month-old infants failed to reach the RDA of 600 mg/d (mean 563 mg/d) while in the older groups, the values were 35% (mean 690 mg/d) and 27% (mean 717 mg/d) respectively. These findings for the youngest group are the result of the relatively low Ca contents of present day infant milk formulas (44–68 mg/100 ml) in comparison with unmodified cow's milk (120 mg/100 ml). The long-term consequences of these low-Ca contents may have important implications.

The mean Fe intakes of infants aged 3–12 months were well above the RDA of 6 mg/d, but nearly 80% of the 12–24 month group failed to reach the RDA of 7 mg/d.

There were no significant social class differences for protein and Ca intakes, but energy and iron intakes tended to be inversely related to social class.

The present findings suggest the possibility that some of the RDAs are too high for this age range, although it may be that some Glasgow infants were potentially deficient, especially in Ca and Fe.

We are grateful to the Scottish Hospital Endowments Research Trust for financial support.

DHSS (1979). *Recommended Daily Amounts of Food Energy and Nutrients for Groups of People in the United Kingdom.* London: H.M. Stationery Office.

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Nitrogen and energy balance studies on pre-term infants. By J. B. MORGAN, R. F. GRIMBLE and S. WHITAKER, *Department of Nutrition* and C. ROLLES, *Department of Child Health, Medical Faculty, University of Southampton, Southampton SO9 5NH*

There is no universally agreed dietary regimen for the pre-term infant (born before 37 weeks gestational age or with a birth weight of less than 2500 g or both) and requirements for nitrogen and energy are speculative. The difficulties of applying theoretical estimates to the practical situation of feeding large volumes or high-energy density milk formulas are well documented (Brooke, 1980). The present study was initiated to establish if a special care unit 'feeding regimen' was adequate when compared with calculated requirements, based on incremental or factorial estimates.

Balance studies over 3 to 9 d periods were performed on thirteen pre-term infants receiving SMA Gold Cap (a humanized cow's milk formula). Daily milk intakes were precisely measured. Total faecal and urine collections were made over the corresponding periods. The faeces relating to the first and last feeds were identified using a coloured faecal marker (carmine or ediol blue) in these milks. All samples were analysed for total N by the Kjeldahl method and energy by bomb calorimetry. Anthropometric data was taken at the beginning and end of each study period.

Subjects were between 33 and 39 weeks gestational age and 2 to 52 d old at the onset of the study. Nude weights ranged from 1450 to 2380 g and supine length from 39 to 48 cm. Weight changes ranged from minus 14·8 to 35·4 g/kg per d and some infants exhibited accelerated or catch-up growth.

Mean (±SD) total energy intake/kg body-weight per d was 472 (54) kJ and metabolizable energy was 400 (50) kJ/kg body-weight per d. Energy digestibility ranged from 74 to 94% and the mean value was 85 (6)%. Mean (±SD) total N intake was 418 (61) mg/kg per d, absorbed N was 355 (87) mg/kg per d and retained N was 287 (109) mg/kg per d. Mean N digestibility was 85% and retention 68%.

Infants with a high level of energy intake (kJ/kg per d) exhibited low percentage digestibilities indicating that high feeding volumes reduced absorptive capacity. However, digestibility tended to improve with gestational age.

No relationship was seen between metabolizable energy or retained N (per caput or /kg body-weight) and gain in weight (g/kg per d) because of the wide variation in these parameters.

Energy and N intakes of some infants may have been inadequate, thus limiting growth.

Brooke, O. G. (1980). *Br. J. Nutr.* **44**, 13.

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A new potent anoretic compound with additional weight-reducing effects.

By D. M. ANDERSON, *Department of Pharmacology, Scientific Development Group, Organon Laboratories Limited, Newhouse, Lanarkshire*

During routine screening of a series of potential anoretic drugs in rats it was noted that Org 6837 (dl-8,9 dichloro-4-exo-hydroxy-11 anti-dimethyl, amino-benzo(b)bicyclo [3,3.1] nonene hydrochloride) not only decreased food intake but caused a greater loss in the animals' weight than could be accounted for by the anorectic effect. This observation has been investigated further using three groups each of eight male Wistar rats (320–340 g) administered 5, 15 or 30 mg Org 6837/kg per d p.o. for 30 d. A further three groups of eight rats were pair-fed (pf) with each of the drug treatments. A similar experiment was carried out simultaneously using a standard anoretic, fenfluramine, at the same dose levels. An additional group of four wholly untreated rats were allowed food *ad lib.* Significance levels between groups were determined using a one-way analysis of variance and two-tailed '*t*' test.

After 30 d of treatment, rats given 30 mg Org 6837/kg per d lost 63 g body-weight while their pair-fed group gained 1·9 g ($P<0·001$). Rats given 15 mg/kg per d lost 12·5 g body-weight compared with a gain of 13 g in the pair-fed group ($P<0·01$), whereas there was no significant difference between pair-fed group (+51 g) and the low dose Org 6837 group (+57 g). All Org 6837-treated rats exhibited a decreased food intake. Fenfluramine-treated animals also reduced food intake and rate of weight gain but there was no significant difference in the body-weight change between fenfluramine-treated groups and their respective pair-fed groups: i.e. 30 mg/kg per d, +19 g *v.* pair-fed, +14 g; 15 mg/kg per d, +51 g *v.* pair-fed, +51 g; and 5 mg/kg per d, +42 g *v.* pair-fed, +23 g. Untreated rats gained 67 g during the experimental period.

The differences in weight change between Org 6837 groups and their respective pair-fed groups are unlikely to be due to an artefact induced by the pair-feeding technique since such differences were not apparent in the simultaneous fenfluramine study. In addition, preliminary toxicological studies have eliminated any obvious case, e.g. glycosuria for the greater weight loss exhibited by the Org 6837-treated animals.

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The effect of concentration of linseed oil supplementation on digestion and microbial synthesis in the stomach of sheep. By O. A. IKWUEGBU and J. D. SUTTON, *National Institute for Research in Dairying, Shinfield, Reading, Berks RG2 9AT*

When Knight *et al.* (1978) fed supplements of 40 g/d linseed oil (LSO) or coconut oil to sheep, there was a marked increase in both flow of microbial N and efficiency of microbial protein synthesis (MPS) but a severe depression in organic matter (OM) digestion in the rumen.

An attempt has therefore been made to define a concentration of LSO at which MPS can be enhanced without the marked reduction in OM digestion. In a randomized block experiment, three sheep fitted with rumen and re-entrant duodenal cannulas were given, in two equal portions at 06.00 and 18.00 hours, a basal ration of 200 g hay and 400 g concentrates daily, providing about 5·8 MJ ME/d and 12·5 g N/d, alone or with supplements of 13, 26 and 40 ml LSO/d.

The flow of duodenal digesta was measured by spot-sampling using chromic oxide paper as the marker. MPS was measured by the diaminopimelic acid (DAPA) technique.

Linseed oil (ml/d)	0	13	26	40	5% LSD
Digestion of OM (DOM)	0·79	0·79	0·76	0·75	0·034
Digestion of OM in stomach:					
Apparent ($ADOM_R$)	0·50	0·47	0·32	0·38	0·066
True ($TDOM_R$)	0·69	0·66	0·55	0·57	0·093
Flow to duodenum:					
Total N (g/d)	11·0	11·8	17·0	14·2	3·62
Microbial N (g MN/d)	7·5	8·1	9·7	6·7	1·94
g MN/kg $ADOM_R$	32	35	59	34	10·1
g MN/kg $TDOM_R$	23	25	35	22	4·7
Protozoa (10^{-4}/ml)	372	211	9	1	115·4

The proportion of DOM apparently digested in the stomach was markedly reduced but not in a regular manner. There was a shift in the digestion of OM from the stomach to the intestines but this just failed to compensate for the reduction in over-all digestion at the highest concentration of oil. The second increment of LSO produced the greatest microbial N flow and efficiency of MPS but the increase was smaller than reported by Knight *et al.* (1978). It is possible that MPS is increased by lower levels of LSO due to the reduction of protozoa but not at higher levels as these also reduce bacterial growth (Van Nevel & Demeyer, 1981). The results emphasize the difficulty of predicting the response of the rumen to oil supplementation of the diet.

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Isotope effect in measuring rumen propionate production in the presence of monensin. By J. B. ROWE, A. DAVIES and A. W. J. BROOME, *Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG*

Prange *et al.* (1978) and Van Maanen *et al.* (1978) have measured the rate of propionate production in cattle given diets with and without monensin (an ionophore), using [1-^{14}C]propionate as a marker. In both studies increases in measured propionate production rates in the presence of monensin were always greater than the observed change in propionate concentration in the rumen. It was concluded that changes in the molar percentage or molar concentration of propionate did not provide a reliable indication of increased propionate production arising from the use of monensin. Mayes *et al.* (1981) found that the estimated production rate of propionate was approximately doubled when [1-^{14}C] as opposed to [2-^{14}C]propionate was used as a marker and showed that this difference could be attributed to the transfer of C between the carboxyl group of propionate and rumen CO_2. This unrepresentative loss of ^{14}C label from the carboxyl position would not alter the relationship between propionate concentration and production rate provided that the degree of equilibration of C between CO_2 and propionate was not affected by the action of monensin.

In a series of experiments in our laboratories twelve mature sheep fitted with permanent rumen cannulas were used to study various aspects of rumen fermentation and twenty sets of separate continuous intraruminal infusions of [2-^{14}C]propionate and $H^{14}CO_3^-$ were carried out. Fourteen of these sets of infusions were in animals receiving diets containing no drug and six were in animals given diets providing 0·5 mg monensin/kg live weight per d. The following measurements were taken: rumen propionate concentration and rate of production, and the proportion of propionate-C derived from rumen HCO_3^--C. The relationship between rumen propionate production and concentration was similar to that described by Leng (1970) irrespective of whether monensin was present in the diet. The proportion of propionate-C arising from HCO_3^--C was significantly ($P<0.001$) higher when monensin was given in the diet (control $22\pm4\%$, monensin $33\pm3\%$). All propionate C derived from $H^{14}CO_3^-$ was in the carboxyl group. It is suggested that this difference occurs through the action of monensin increasing propionate production, principally through the succinate pathway where there is a greater possibility for inclusion of HCO_3^--C through the processes of carboxylation and decarboxylation than occurs in the acrylate pathway. These results raise questions on the usefulness in [1-^{14}C]propionate as a marker in studies involving the use of monensin. The pathways by which high levels of CO_2-C incorporation into propionate may occur are being investigated.

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The effect of Synperonic NP9 upon ciliate-free and faunated sheep. By J. MARGARET EADIE and W. J. SHAND, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The teric compound nonyl phenol ethoxylate (Synperonic NP9, ICI) was reported by Australian workers to defaunate both sheep and cattle given low protein, high carbohydrate diets (Bird *et al.* 1979). Better performance in the treated animals was attributed to the absence of rumen ciliate protozoa. As small ciliate populations, mainly *Entodinium* spp, were involved, it seemed of interest to examine the effect of Synperonic upon control sheep with and without a large rumen ciliate population. Three-year-old sheep on a roughage–concentrate diet (2:1) had been managed from 3 d of age as decribed by Eadie (1979). Synperonic (0·55 g/kg body-weight) was administered to two ciliate-free sheep, two in which only *Entodinium* spp were present and two in which a mixed Type A or Type B population was established (cf. Eadie, 1979).

Food consumption and water intake were monitored. Rumen fluid samples were taken at least once daily for 4 d and thereafter at longer intervals. All samples were examined microscopically and some viable bacterial counts were made. Rumen volatile fatty acid proportions, ammonia nitrogen concentrations and pH values were determined.

Many ciliates were killed but 2 h after treatment live organisms could still be found and by the 6th d the ciliate population had reverted to normal. However, both phytoflagellates and *Oscillospira* took up to 8 weeks to re-appear. Tests of Synperonic toxicity in vitro also showed remarkable ciliate survival.

Appetite was depressed for 4 d in four animals. All sheep showed a consistent change in rumen VFA proportions; an increase in propionate and decrease in acetate being most marked after 2 d. Rumen pH values were lower than normal for the food intake. There was reduced water intake and urine production in some animals and this, coupled with possible depressed gut movement and lowered pH, could account for the exceptionally high ammonia values obtained. Total bacterial numbers were depressed initially but consistent changes in proportions of bacterial types were not seen.

These results showed that on a conventional diet the effect of Synperonic upon both ciliate-free and faunated animals was similar and suggest that any benefit accruing from the administration of Synperonic is unlikely to be due solely to an absence of ciliates.

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Zinc deficiency increases placental prostaglandin synthesis from arachidonic acid. By S. C. CUNNANE, *Rowett Institute, Aberdeen AB2 9SB*

Excessive placental haemorrhaging and high foetal mortality at parturition occur characteristically in zinc deficient pregnant rats. These features may be related to compromised placental perfusion resulting in intrapartum foetal anoxia. The possibility that these effects were due to abnormal placental prostaglandin (PG) synthesis from arachidonic acid (AA; 20:4ω6) was therefore considered.

Second parity Hooded Lister rats (Rowett strain) were fed on semi-synthetic diets containing Zn at 20 ppm (control), 5 ppm (chronic Zn deficient), or 10 ppm for 2 weeks reduced to 0·5 ppm for 1 week (acute Zn deficient). On day 22 of gestation 200 mg of placental tissue was incubated in Krebs-Henseleit buffer with [1-^{14}C]AA for 30 minutes at 37°. The reaction mixture was then acidified, and the lipids extracted. PGs were separated by thin layer chromatography (Merck 5735 plates) and the radioactivity counted in the bands corresponding to AA, PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$.

Zn deficiency (both chronic and acute) significantly increased synthesis of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ by 60–190% ($P<0.05$). Synthesis of PGE_2 was not affected by chronic Zn deficiency but was significantly increased in acute Zn deficient rats to 157% of control ($P<0.01$).

It was concluded that Zn deficiency caused enhanced placental conversion of exogenous AA to PGs, an effect which would contribute to decreased placental perfusion through PG-induced vasoconstriction. Combined with the previous observation (Cunnane, 1981) that Zn deficiency at parturition causes significantly decreased uterine synthesis of PGE_2, $F_{2\alpha}$ and 6-keto-$F_{1\alpha}$, these results suggest that foetal hypoxia and uterine hypocontractility may contribute to the enhancement of foetal mortality in Zn deficiency.

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Effects of frozen turnips and mineral supplements on incisor tooth loss in sheep. By the late D. BENZIE, R. N. B. KAY and J. C. GILL, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Broken mouth, the loss of incisor teeth, commonly occurs among breeding ewes on upland and hill farms. Benzie & Cresswell (1962) found that the condition was often associated with the feeding of turnips in winter; although the incisor alveolar bone became severely eroded in affected sheep the teeth and skeleton were quite well mineralized so that mineral deficiency did not seem to play an important role. On the other hand Gunn, while agreeing that the grazing turnips accentuated broken mouth (Gunn, 1970), had also shown that a calcium-phosphorus supplement improved the firmness and permanence of the incisor teeth of ewes at hill pasture and suggested that gradual mineral depletion caused weakening of the aveolar bone (Gunn, 1969). As field turnips are sometimes frozen hard in winter it was decided to see if freezing was of importance.

Forty-eight ewe lambs were divided into four groups. For 7 to 8 weeks in five successive winters they were held in an open shelter and given turnips (6 kg/head) either frozen (−10°) or unfrozen for 4 d/week, and ground maize (0·4 kg/head) supplemented or not with ground limestone (50 g/kg) together with hay to appetite for 3 d/week. The sheep ate the frozen turnips eagerly, scraping off long flakes with their incisors and leaving almost no residue. At other times the sheep were pastured as a breeding flock.

The teeth were periodically examined visually and radiographically. By 5 years of age thirteen of the forty-four surviving sheep had broken mouths while loose teeth and deep periodontal pockets were common (see Table). The incidence of loose and lost teeth was significantly greater in the groups receiving frozen turnips, an effect undiminished by the mineral supplement. This suggests that mechanical trauma, possibly together with chilling of the alveolar bone, was responsible for the group differences.

Sheep group ...	A	B	C	D	A+C	B+D	Statistical significance of differences between groups A+C and B+D
Winter treatment ...	F	M	FM	Neither			
	Broken mouth* (no. of sheep)				>15% looseness† (no. of sheep)		
September 1968	0 (12)	0 (12)	0 (12)	0 (12)	4 (24)	0 (24)	NS
October 1969	1 (12)	0 (11)	0 (12)	0 (11)	8 (24)	3 (22)	NS
October 1970	3 (11)	0 (11)	2 (12)	0 (11)	15 (23)	3 (22)	$P<0.001$
					>25% looseness (no. of sheep)		
March 1971	5 (11)	2 (11)	5 (12)	1 (10)	11 (23)	3 (21)	$P<0.05$

NS, not significant; F, frozen turnips; M, mineral supplement; Neither, unfrozen turnips, no supplement.

*One or more of six central incisors missing.

†Score for four central incisors where 20%, slightly loose; 40%, loose; 60%, very loose; 80%, held by soft tissue only; 100%, tooth missing.

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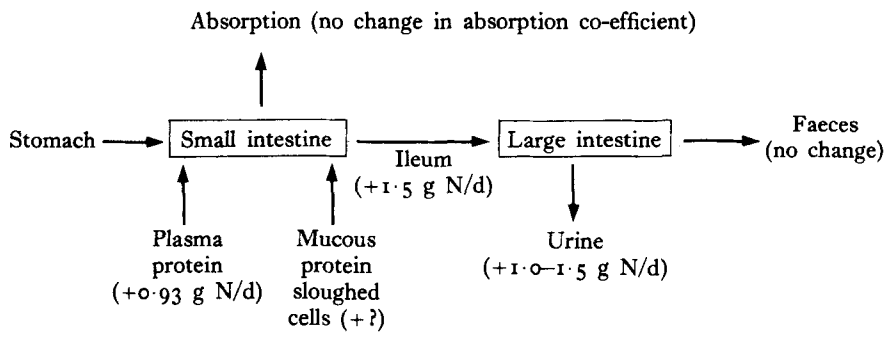
Nitrogen digestion in sheep infected with intestinal parasites. By D. P. POPPI, J. C. MACRAE and W. CORRIGALL, *The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and R. L. COOP, *Moredun Research Institute, Gilmerton Road, Edinburgh EH17 7JH*

Lambs infested with the small intestine roundworm *Trichostrongylus colubriformis* excrete more urinary N and exhibit a poorer N retention than do pair-fed control lambs (Steel *et al.* 1980). These effects were studied in ten 4-month-old lambs prepared with simple cannulas into the rumen, duodenum and ileum. The lambs were paired on a live weight basis and were pair-fed on complete ruminant diet AA6. One lamb in each pair was dosed daily for 14 weeks with 2500 *T. colubriformis* larvae. On two occasions, i.e. during weeks 0–2 and 5–7 after the start of dosing, measurements were made of N balance (5 d collections), amounts of N passing the ileum (using ^{103}Ru-P and ^{51}Cr-EDTA dual phase markers in two separate 24 h collections), true digestibility and absorption of protein between the duodenum and the ileum (using ^{35}S labelled bacteria; Salter & Smith, 1977) and leakage of plasma protein into the small intestine (using $^{51}CrCl_3$; Holmes & MacLean, 1971).

In weeks 0–2 there were no differences between infected and control animals for any of these measurements. However, in weeks 5–7 corresponding with the maximum output of faecal eggs, the infected lambs had a urinary N excretion (1–1·5 g/d; $P<0·1$), ileal N flow rate (total N 1·5 g/d, $P<0·05$; supernatant N 1·7 g/d, $P<0·01$) and leakage of plasma protein into the small intestine (0·93±0·32 g N/d, $P<0·05$). There were no differences in the digestibility of ^{35}S-labelled bacteria (0·709±0·028 *v.* 0·709±0·034) or apparent N digestibility.

These results can be used to formulate the flow diagram given in Fig. 1. The extra ileal N was sufficient to account for the increased urinary N in the infected lambs. This ileal N appeared to be more associated with endogenous protein entering the small intestine than with malabsorption. If the ^{35}S absorption data can be assumed for all proteins entering the small intestine, then the extra 1·5 g ileal N would represent an increase of perhaps 5 g of endogenous N entering the small intestine. Since less than 1 g of this was plasma protein leakage, more than 4 g N must have come from other sources, such as cell sloughing and mucin secretions.



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The production of 5,8,11 eicosatrienoic acid, (20:3ω9) in the EFA deficient cat. By J. P. W. RIVERS and T. L. FRANKEL, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

We have previously reported to the Society that cats fed diets containing vegetable oils rich in linoleic acid (18:2ω6) have high levels of linoleic acid in tissue lipids, accompanied by depleted levels of arachidonic acid (20:4ω6) and other ω6 fatty acids related to linoleic acids (Rivers, Sinclair *et al.* 1976).

We have associated this phenomenon with a lack of significant Δ6 or Δ8 desaturase activity in the cat, for which we have presented supporting evidence (Rivers, Hassam *et al.* 1976). We here report on further long-term studies involving feeding diets containing safflower seed oil, evening primrose oil, and hydrogenated coconut oil.

These experiments confirm the lack of significant Δ6 and Δ8 desaturase activity but indicate that Δ9, Δ5 and Δ4 desaturases are present. In all the experiments the Δ5 and Δ4 desaturase activity were slight compared to the rat, and sometimes undetectable.

In two separate studies, when two young cats were transferred from a diet of proprietary cat foods to experimental diets containing hydrogenated coconut oil, 20:3ω9 was detected in tissue lipids. However, the amount was small (<0·5% of fatty acids) in every tissue lipid fraction examined, even in animals fed on the HCO diet for two years. In contrast a fatty acid we identify as 5,11,14 eicosatrienoic acid was at levels of 2% or more in cats given all experimental diets.

In a final experiment, cats fed on a safflower seed oil diet were transferred to an HCO diet for 12 months. No 20:3ω9 was detected in the tissue lipids of these animals. A probable reason is that tissue levels of 20:2ω6 remained sufficiently high throughout the experiment that production of 20:3ω9 was inhibited, by competition between ω6 and ω9 fatty acids for the Δ5 desaturase enzymes.

The exact pathway of biosynthesis of 20:3ω9 by the cat remains obscure. It may be that, as in other mammals, it involves the Δ6 desaturation of 18:1ω9 or the Δ8 desaturation of 20:1ω9, but with enzyme activities so low that we have failed to detect them in our other experiments. Alternatively the pathway could proceed by the Δ4 desaturation of 16:1ω9. Though Δ4 desaturase activity is low in the cat, we do find as evidence that the enzyme exists, and HCO fed cats do have small amount of 16:1ω9 in their tissue lipids, presumably produced by the chain shortening of endogenously synthesized 18:1ω9.

Whatever the pathway, the biosynthesis of 20:3ω9 by the cat suggests that production of 20:4ω6 from 18:2ω6 may also occur. However, these results also suggest a rate of production which is so low, that we regard it as of no nutritional significance, and still predict a dietary requirement for 20:4ω6 by the cat.

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