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## **PROCEEDINGS OF THE NUTRITION SOCIETY**

### **ABSTRACTS OF COMMUNICATIONS**

*The Four Hundred and Twenty-fifth Meeting of the Nutrition Society was held in the Rupert Beckett Lecture Theatre, University of Leeds, Leeds on Monday and Tuesday, 24/25 March 1986, when the following papers were read:*

**Diurnal influences on the metabolic effects of two types of *Escherichia coli* endotoxin in rats.** By JENNIFER WAN and R. F. GRIMBLE, *Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Diurnal variations occur in the mortality of animals exposed to bacterial endotoxin (Ucar *et al.* 1983). We have found that some preparations of *Escherichia coli* endotoxin (phenol and trichloroacetic acid (TCA) extract) are hypothermic while butanol extracts cause a fever. In the present study, diurnal differences in the response to hypo- and hyperthermic varieties of endotoxin (TCA extract, strain 055:B5, Difco Labs (ED) and butanol extract, strain 0127:B8 (ES) respectively) were examined by injection into rats adapted to normal and reversed daily lighting cycles.

Male Wistar rats ( $178 \pm 2$  g) were given standard laboratory chow and adapted to normal (N) (06.00–18.00 hours) or reversed (R) (18.00–06.00 hours) light cycles. At 09.00 hours control and test animals from each group ( $n$  5) were injected intraperitoneally with non-pyrogenic saline (9 g sodium chloride/l) or ED or ES (0.8 mg/kg body-weight). Rectal temperatures ( $t$ ) were recorded hourly for 5 h and animals decapitated 19 h later. Blood and tissues were collected for analysis. As food intakes were reduced following endotoxin injections, groups of saline-injected rats (PF), pair fed with each endotoxin group, were included in the study.

Group	Normal lighting (N)				Reversed lighting (R)			
	$t_0$	$t_2$	$t_5$	$\Delta t_0-t_2$	$t_0$	$t_2$	$t_5$	$\Delta t_0-t_2$
Saline <i>ad lib.</i>	39.4	39.1	38.9	-0.3	40.3	39.2†	40.0	-1.1‡
ED	39.6	36.9†*	38.6†	-2.7	40.4	36.8†	37.7†*	-3.6
ES	39.3	39.2	39.8*†	-0.2	40.4	38.3†	39.5	-2.1‡
	Saline PF	ED	Saline PF	ES	Saline PF	ED	Saline PF	ES
Food intake (g/d)	1	1	8.8	8.8	1	1	3.8	3.8
Serum Zn ( $\mu\text{g/ml}$ )	2.52	1.36*	2.08	1.50*	2.40	1.16*	1.84‡	1.12*‡
Liver protein (g)	1.16	1.92*	1.27	2.11*	1.15	1.92*	1.19	1.75*‡
Muscle protein (%)	17.4	16.2*	17.1	15.9*	17.8	15.7*	18.1‡	16.7*

Significantly different from corresponding saline group values: \* $P < 0.05$ .

Significantly different from  $t_0$  value: † $P < 0.05$ .

Significantly different from normal lighting values: ‡ $P < 0.05$ .

While the effect of both endotoxins on tissue protein content was independent of diurnal factors, serum zinc and body temperature responses showed diurnal modification. The fever-promoting endotoxin ES became hypothermic in R animals. The hypothermic endotoxin ED produced a more prolonged effect in R than in N animals. Similarly, the depression of serum Zn was greater in the former. While Tocco (1983) suggested that fever and serum Zn changes during infection are unrelated, our results suggest a possible link between the degree of hypothermia and Zn metabolism.

Tocco, R. J., Kahn, L. L., Kluger, M. J. & Vander, A. J. (1983). *American Journal of Physiology* **244**, R368–R373.

Ucar, D. A., Tocco, R. J. & Kluger, M. J. (1983). *Proceedings of the Society for Experimental Biology and Medicine* **173**, 319–323.

**The interrelation between glycogen, water and potassium.** By A. W. JOHNSON, *Department of Medicine, St James's University Hospital, Leeds* and S. T. F. CHAN, C. R. KAPADIA and H. A. F. DUDLEY, *Surgical Unit, St Mary's Hospital Medical School, London W2*

During glycogenesis, 2–3 g water and 0.45 mmol potassium are obligated to each gram of glycogen and thus are accumulated intracellularly (Fenn & Haegle, 1940; Bergstrom & Hultman, 1972; Chan *et al.* 1982). These effects can cause a weight gain of approximately 1.5 kg when starved patients are refeed with glucose-containing solutions. However, the exact relation between glycogen, water and K within the cell is not known. Therefore the aim of the present study was to investigate the movement of water between body compartments during glucose refeeding with and without K.

Ten volunteers were starved for 4 d before refeeding for 6 h with glucose alone, glucose with K (six volunteers, paired study), or glucose followed by glucose with K (four volunteers). Plasma and urine sodium, K and glucose concentrations and osmolality were measured before and during refeeding. Substrate oxidation was calculated from respiratory gas analysis and urinary nitrogen excretion.

	Glucose				Glucose + K			
	Prefeeding		Postfeeding		Prefeeding		Postfeeding	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma Na (mmol/l)	135.5	0.6	134.7	0.5	134.4	0.9	140.1*	1.1
Osmolality (mosmol/l)	281	2	281	2	283	1	304*	1

\* $P < 0.05$ .

There was no change in plasma Na concentration or osmolality during glucose only refeeding; however, these two variables increased significantly when K was available (Table). Fat oxidation was predominant during all stages of refeeding, although some glucose oxidation was detected towards the end of refeeding. Both Na and N excretion were reduced during refeeding.

These results suggest that glycogen storage occurs during glucose refeeding at the expense of glucose oxidation following short-term acute starvation. Furthermore, there is no overall movement of water between body compartments during glucose only refeeding, only when K is available is there any intracellular accumulation of water during glycogenesis. Thus it is possible that it is K which obligates intracellular water and that glycogen is osmotically relatively inert.

Bergstrom, J. & Hultman, E. (1972). *Journal of the American Medical Association* **221**, 999–1006.

Chan, S. T. F., Johnson, A. W., Moore, M., Kapadia, C. R. & Dudley, H. A. F. (1982). *Human Nutrition: Clinical Nutrition* **36C**, 223–232.

Fenn, O. E. & Haegle, L. F. (1940). *Journal of Biological Chemistry* **136**, 87–101.

**Undernutrition in Alzheimer's disease.** By S. SINGH<sup>1</sup>, A. W. JOHNSON<sup>1</sup>, G. P. MULLEY<sup>2</sup> and M. S. LOSOWSKY<sup>1</sup>, *Departments of <sup>1</sup>Medicine and <sup>2</sup>Medicine for the Elderly, St James's University Hospital, Leeds*

Alzheimer's disease is the most common type of dementia; its aetiology is unknown and there is no current treatment. It has been thought clinically that patients suffering from this disease are undernourished compared with other long-stay patients, although no hard evidence is available to substantiate this observation. Therefore the aims of the present study were to determine if Alzheimer's patients are malnourished and, if so, to investigate the cause.

Three groups of elderly female in-patients of comparable age and mobility were studied: (1) an Alzheimer group (ALZ, *n* 29), which were selected by Hachinski scores, (2) a non-demented group (ND, *n* 25) and (3) a multi-infarct dementia group (MID, *n* 20).

A nutritional assessment was performed using body-weight, triceps and biceps skinfolds and plasma concentrations of several nutritional indices (albumin, calcium profile, haemoglobin, mean corpuscular volume, folate, vitamins B<sub>12</sub>, A and E) as the measured variables. Body fat was calculated using standard equations (Durnin & Womersley, 1974). The results of the body composition analysis are shown in the Table.

	ALZ		ND		MID	
	Mean	SD	Mean	SD	Mean	SD
Body-weight (kg)	41.7	6.9	53.1	8.8	48.4	10.7
Body fat (kg)	9.2*	3.7	15.6	5.0	12.4	5.9
Fat free mass (kg)	32.5*	4.1	37.5	5.2	36.0	6.5

\* $P < 0.05$  (Mann Whitney U test).

Alzheimer's patients were significantly lighter (Table) than the comparable groups of ND and MID patients; this was mainly attributable to lower fat reserves. A retrospective study showed that the Alzheimer's group were the only ones in whom significant weight loss had occurred. All the plasma nutritional indices were similar between the groups.

An assessment of recent food intake and of faecal fat excretion did not support dietary intake or malabsorption as being contributory factors to the observed weight loss.

The reasons for the change in body composition of Alzheimer's patients remain unknown.

Durnin, J. V. G. A. & Womersley, J. (1974). *British Journal of Nutrition* 32, 77-97.

**Use of a computer-administered 'healthy eating' quiz for data collection and health promotion.** By A. J. BRAY and J. R. KEMM, *Department of Community Medicine, Central Birmingham Health Authority, Queen Elizabeth Hospital, Birmingham B15 2TJ*

The extreme difficulty of collecting reliable data on nutrient intake has led many to investigate the use of food questionnaires as a method for monitoring food-intake patterns (Block, 1982). Quizzes on eating patterns have also been used as aids to health promotion, most recently by the Joint Advisory Committee on Nutrition Education, (1985). Computers have been used both as dietary data collection devices and as aids to dietary counselling (Slack *et al.* 1976). We have attempted to combine these approaches and produce a computer-administered quiz with the dual objectives of (1) raising awareness of food and health issues and (2) providing information on eating patterns within the health-district population.

Participants are invited to answer a short set of multiple choice questions on food habits. The responses given are stored by the program with information on the participant's age and sex for subsequent analysis. The program responds with an individualized printout giving 'tips on healthy eating'. The program has a library of possible tips. The selection of tips for printing is determined in part by the response to the quiz so that the tips printed for each participant are relevant to their own eating patterns, and in part by a random process so that two participants who give identical replies are most unlikely to receive identical printouts. Our early experience at health fairs and exhibitions suggests that the program is effective in attracting people to the stand. The printout facilitates discussions with health-promotion staff on the stand by providing a talking point and also prolongs interest in the topic since participants are likely to compare and discuss their individual printouts. It must be emphasized that the printout is not intended to be a substitute for individual dietary counselling where needed.

The quiz questions can either be presented to the participants on the screen, responses being entered directly into the program by pressing the appropriate keys, or the answers marked off on a printed sheet and keyed in by a technician. This second method of presenting the quiz is more appropriate for data collection since there is more control over data entered into the system. We propose to try and use data collected by the quiz first to identify target groups (age and sex groups) for 'health promotion' activities, second to improve the relevance of health promotion messages by identifying the commonest 'unhealthy' eating patterns and third to monitor changes in eating patterns of the district population. Clearly data collected in this way have to be interpreted with great caution until we know whether the responses to the quiz reflect the eating patterns of the participants and whether the eating patterns of a self-selected group of quiz participants is representative of the district population.

The program is written in BASIC and is currently run on Apricot and Sirius machines, but it could easily be adapted for any microcomputer which has a disc drive and printer.

Block, G. (1982). *American Journal of Epidemiology* 115, 492-505.

Joint Advisory Committee on Nutrition Education (1985). *Eating for a Healthier Heart*. London: British Nutrition Foundation and Health Education Council.

Slack, W., Porter, D., Witschi, J., Sullivan, M., Buxbaum, R. & Stare, F. J. (1976). *Journal of American Dietary Association* 69, 514-517.

**A survey of infant feeding practice by Afro-Caribbean mothers in Birmingham.** By J. R. KEMM, J. DOUGLAS and V. SYLVESTER, *Department of Social Medicine, University of Birmingham*

Recent studies have provided information on changing infant feeding practice in the UK (Martin & Monk, 1982; Taitz, 1983) and differences in practice between ethnic groups have been reported (Jones & Belsay, 1977; Treuherz *et al.* 1982).

The sampling frame for our surveys consisted of all Afro-Caribbean infants born to mothers resident in Birmingham. Children born to mothers under the age of 18 years, children with congenital malformations and multiple births were excluded. The mothers of the children born in specified weeks were visited and asked for an interview.

The pooled results of four studies covering 131 infants in which the mothers were interviewed between 3 and 15 months after the birth are presented. The incidence of breast feeding defined as 'ever having put to the breast' appears to be rather higher (91%) in our sample than in other recent surveys, but the number who continued to breast feed estimated by a life table technique fell sharply with time, being 78% at 1 week, 64% at 4 weeks, 43% at 8 weeks, 34% at 3 months and 24% at 6 months so that after 2 months the number breast feeding in our study appeared to be similar to that found in other studies.

*Comparison of maternal characteristics for those breast feeding for less than 1 month or not at all and those breast feeding for 1 month or more*

	Less than 1 month		1 month or more		Statistical significance
	Yes	No	Yes	No	
Owner/occupier	6	41	29	55	$P < 0.02$
Receiving single parent allowance	23	23	25	59	$P < 0.05$
Age less than 25 years	26	21	39	45	NS
In paid employment	10	28	37	56	NS
Mother born in UK	28	19	47	37	NS

NS, not significant.

Mothers in council-rented accommodation and single mothers were less likely than other mothers to continue breast feeding for more than 1 month, confirming other studies. On the other hand, age of mother, age of leaving school, employment status and living in a large household were not related to the duration of breast feeding in this study. It appears that the factors associated with successful breast feeding in Afro-Caribbeans in Birmingham may be rather different than those in other sections of the UK population.

Jones, R. A. K. & Belsay, E. M. (1977). *Social Science and Medicine* 11, 175-179.

Martin, J. & Monk, J. (1982). *Infant Feeding 1980*. Publication of the Office of Population Census and Surveys. London: H.M. Stationery Office.

Taitz, L. S. (1983). *British Medical Journal* 287, 648.

Treuherz, J., Cullinan, T. R. & Saunders, D. I. (1982). *Human Nutrition: Applied Nutrition* 36A, 2811-286.

**Acute hypoglycaemia inhibits increased luteinizing hormone pulsatility associated with weaning in postpartum beef cows.** L. M. RUTTER and J. G. MANNS (introduced by B. LAARVELD), *Department of Veterinary Physiology Science, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada*

Nutrition and suckling affect reproductive function in cattle (Dunn & Kaltenbach, 1980; Edgerton, 1980); however, the mechanisms which interrelate those factors remain to be elucidated. We utilized phlorizin, which inhibits glucose uptake from renal tubules, to determine if acute hypoglycaemia alters pulsatile luteinizing hormone (LH) secretion initiated by weaning.

Cross-bred Hereford cows, fitted with indwelling jugular catheters at  $32 \pm 2.5$  d postpartum (experimental day 0), were infused continuously with phlorizin (PLZ: 3 g/d;  $n$  9) or saline (9 g sodium chloride/l; weaned control, WC,  $n$  10; suckled control, SC,  $n$  8) from experimental day 2 to day 4. Calves from PLZ and WC groups were removed after the sampling period on day 1 and kept separate through to day 4, except for a 1 h sucking period on day 3. Blood was collected at 10-min intervals for 6 h on days 1 and 4 and serum was assayed for LH concentration. (LH pulse = two consecutive values  $\geq 40\%$  above basal.) Plasma insulin, glucose and free fatty acid (FFA) concentrations were determined in samples collected every 2 h for 8 h on day 1 through to day 4; samples for progesterone were collected on days -1, 1 and 3, and twice weekly for 3 weeks thereafter.

By day 3, the PLZ group had a mean urinary secretion of 567 (SE 40) g glucose/d compared with  $< 3.0$  g/d in the WC and SC groups ( $P < 0.001$ ). Plasma glucose concentrations were depressed ( $P < 0.05$ ) in the PLZ group on day 2 compared with pre-infusion levels (639 (SE 17) *v.* 690 (SE 24) mg/l) but were similar to pre-infusion levels and those found in the SC group by days 3 and 4. Plasma insulin and FFA concentrations remained stable over the experimental period in both PLZ and SC groups. In contrast, the WC group had elevated plasma glucose (74.7 (SE 10) and 745 (SE 12) *v.* 680 (SE 16) mg/l;  $P < 0.05$ ) and insulin (27.1 (SE 2.3) and 29.6 (SE 2.8) *v.* 15.3 (SE 1.8)  $\mu$ U/ml;  $P < 0.05$ ) on days 3 and 4 compared with day 1, while FFA concentrations declined from day 1 to day 4 (0.53 (SE 0.05) to 0.38 (SE 0.07) mM;  $P < 0.05$ ).

Comparing days 1 to 4 across all cows, mean LH release (0.36 (SE 0.1) to 1.16 (SE 0.17) ng/ml,  $P < 0.001$ ), pulse frequency (1.7 (SE 0.1) to 3.1 (SE 0.2) pulses/6 h,  $P < 0.001$ ) and pulse amplitude (1.46 SE 0.11 to 3.45 SE 1.47 ng/ml,  $P < 0.01$ ) increased. However, both PLZ and SC groups had more ( $P < 0.01$ ) small (0.50 to 1.50 ng/ml) and fewer medium (1.51 to 2.50 ng/ml) pulses on day 4 than did the WC group. Furthermore, the number of small, medium and large ( $> 2.50$  ng/ml) pulses did not change in the PLZ group, but the WC group had fewer small pulses and more medium and large pulses ( $P < 0.005$ ) on day 4 compared with day 1. Despite altered LH pulsatility, the number of cows ovulating (progesterone  $\geq 1.0$  ng/ml) in response to weaning or by 55 d postpartum was similar for the PLZ and WC groups (4 and 2 of 9 *v.* 5 and 3 of 10).

The present study has shown for the first time that acute hypoglycaemia can alter the pattern of LH release in the postpartum beef cow and suggests a mechanism whereby nutrition and reproduction may be interrelated.

**The effects of energy intake in pregnancy and lactation on the reproductive performance of the sow and growth of the litter.** By PAULINE A. LEE and W. H. CLOSE, *Animal and Grassland Research Institute, Church Lane, Shinfield, Reading RG2 9AQ*

As it is known that variations in feed intake in pregnancy or lactation, or both, influence subsequent reproductive performance (Cole, 1982), an experiment was designed to study the effects of varying dietary energy intake in pregnancy and lactation on the productivity of the sow and growth of the litter. The experiment was designed in a 2×2 factorial arrangement involving two feeding levels in pregnancy anticipated to promote 30 (high) and 10 (low) kg net maternal gain, with two energy intakes in lactation, one representing a conventional intake (high) and the second 25% below this (low). The piglets were weaned at 3 weeks of age and the sows continued on the experiment for at least three parities. Results are given for sixty-five, forty-two and twenty-two animals on parities 1, 2 and 3 respectively.

*The influence of feeding level during pregnancy and lactation on both litter weight gain and the energy requirement for piglet growth during a 21-d lactation\**

Parity:	Feed intake: Pregnancy . . .		Lactation . . .		SEM
	High	High	Low	Low	
		High	Low	High	Low
	Litter weight gain (kg)				
1	29.6	27.6	21.9	26.5	2.53
2	30.5	29.3	32.4	25.2	2.59
3	38.4	32.8	30.6	34.9	4.85
	Energy requirement for piglet growth (MJ/kg)				
1	328	341	396	330	311
2	357	351	367	381	373
3	301	309	390	316	377

\*Differences between values were not statistically significant.

At each parity the total weight of the litter at birth was independent of feeding level during pregnancy, but it tended to increase with parity. The application of the feed was important since a low feeding level in lactation, coupled with a low energy intake in the following pregnancy, caused a significant reduction in litter birth weight in parities 2 and 3. The weight gain of the litter during sucking was not significantly influenced by maternal energy intake in either pregnancy or lactation. The daily dietary energy intake requirement per kg piglet weight gain was similar for all treatments over all parities. Thus the sows at the lower feed intakes during lactation needed to utilize more body reserves during the sucking period and this resulted in a significant loss in body condition at weaning.

Cole, D. J. A. (1982). In *Control of Pig Reproduction*, pp. 603–620 [D. J. A. Cole and G. R. Foxcroft, editors]. London: Butterworths.



**The effect on birth weight of a high-protein, low-carbohydrate diet during pregnancy.** By M. CAMPBELL-BROWN, *Northwick Park Hospital, Harrow, Middlesex HA1 3UJ*, F. D. JOHNSTONE, *Department of Obstetrics and Gynaecology, Edinburgh University* and J. F. KERR GRIEVE, formerly *Motherwell Maternity Hospital, Motherwell, Lanarkshire*

Women during pregnancy continue to be advised to alter their diets, usually for two reasons, either to ensure their nutrient intake or to control weight gain but the effect of this advice on pregnancy outcome has never been properly examined. Over a period of 30 years, women in the Scottish town of Motherwell near Glasgow were advised to eat a low-carbohydrate, high-protein diet which contained 0.45 kg meat a day. The quality rather than the quantity of the diet was emphasized and the women ate to satiety. How the women interpreted the diet is shown in Table 1 and compared with that of pregnant women in Aberdeen.

Table 1. *Mean daily intakes of energy, protein and carbohydrate (from 7-d weighed intakes)*

	n	Energy		Protein (g)	Carbohydrate (g)
		MJ	kcal		
Motherwell	18	6.0	1443	88	97
Aberdeen*	140	8.7	2089	71	241

\*Campbell *et al.* 1982.

The energy content of the diets in Motherwell was only 68% of that of the usual Scottish diet eaten by the women in Aberdeen and the protein content was 24% greater. Maternal weight gain during pregnancy reflected the lower energy intake, it was 0.25 kg/week compared with 0.44 kg/week found for women in Aberdeen. Intrauterine growth was also slowed and the distribution of birth weight is shown in Table 2 as percentiles derived from the Aberdeen standards from which Glasgow births differ only minimally (Forbes & Smalls, 1983).

Table 2. *All singleton births to primigravidae over a 12-month period*

Percentile range . . .		<5	5- <10	10- <25	25- <50	50- <75	75- <90	90- <95	≥95	Total
Motherwell	n	24	21	69	82	58	17	8	8	287
	%	8	7	24	29	20	6	3	3	100
Expected distribution	n	14	14	43	72	72	43	14	14	286
	%	5	5	15	25	25	15	5	5	100

$\chi^2 = 50.7, P < 0.001$  (goodness of fit test).

It is evident that for the Motherwell women there has been a profound slowing of intrauterine growth with the whole birth weight distribution shifted to the left; their lower pregnancy weight-gain supports the possibility that this was due to the use of the low-carbohydrate, high-protein diet during pregnancy. To determine whether the diet had influenced perinatal survival was not possible with such small numbers but the Scottish Registrar General's figures for perinatal mortality over the years when the diet was used, show no adverse effects on the births in Motherwell.

Campbell, D. M., Campbell-Brown, B. M., Jandial, L. & MacGillivray, I. (1982). *Proceedings of the Nutrition Society* 41, 30A.

Forbes, J. F. & Smalls, M. J. (1983). *British Journal of Obstetrics and Gynaecology* 99, 279-303.

**The body composition of nutritionally-restricted ovine fetuses compared with younger fetuses of the same weight.** By J. J. ROBINSON, I. McDONALD, K. PENNIE and R. I. SMART, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The body composition of twenty-one Suffolk × Finn Dorset fetuses that were restricted in size by low-plane feeding of their dams from 90 d of gestation to slaughter on days 132 to 136 of gestation was compared with that for eighteen unrestricted younger fetuses (mean gestational age 120 d) of similar weight. The weights of the major tissues and organs were obtained by physical dissection and the chemical constituents were determined on freeze-dried subsamples obtained following the separate mincing of each fetus. Treatment means for the weights of a selection of body organs, muscles and chemical constituents are given in the Table.

	Nutritionally-restricted (NR)	Age-restricted (AR)	NR:AR	Significance of difference of ratio from 1.0†
No. of litters	7	6		
Mean litter size	3	3		
Mean fetal age (d)	134	120		
Fetal weight (g)	2310	2350	0.98	—
Organ weights (g):				
Brain	48	37	1.29	$P < 0.001$
Heart	16	14	1.14	$P < 0.05$
Lungs and trachea	84	86	0.97	NS
Liver	54	79	0.70	$P < 0.001$
Muscle weights (g):				
Longissimus dorsi	22	24	0.88	NS
Gastrocnemius	5.5	5.6	0.99	NS
Chemical constituents (kg):				
Dry matter	0.45	0.41	1.10	$P < 0.001$
Crude protein	0.32	0.28	1.12	$P < 0.001$
Fat	0.038	0.043	0.88	NS
Calcium	0.023	0.019	1.21	$P < 0.001$
Phosphorus	0.013	0.011	1.19	$P < 0.01$

†NS, not significant.

Significance tests carried out after adjustment by analysis of covariance to allow for the small differences between NR and AR in fetal weight.

The ratios, weights of the body constituents of the NR fetuses: those of the AR fetuses, are in agreement with the finding, based on the chemical composition of small fetuses from large litters, that for growth-retarded fetuses some organs (heart, brain) and body constituents (protein, Ca, P) are more developed and others (liver, fat) less developed than an overall allometric relation would indicate (McDonald *et al.* 1979).

McDonald, I., Robinson, J. J., Fraser, C. & Smart, R. I. (1979). *Journal of Agricultural Science, Cambridge* 92, 591–603.

**Glycine insufficiency in pregnancy assessed by urinary pyroglutamic acid excretion.** By A. A. JACKSON\*, C. PERSAUD, J. MCDERMOTT and B. DE BENOIST, *Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7, Jamaica*

The flow of nitrogen from isotopic glycine to urea is limited in preterm infants and during the 3rd trimester of pregnancy. We interpreted this as indicating that the large demand for glycine made by the rapidly growing fetus exceeds the ability of the body to produce glycine endogenously. In pyroglutamic aciduria, an inborn error of metabolism (deficiency of glutathione synthase (*EC* 6.3.2.3)) results in an increased urinary excretion of pyroglutamic acid. We reasoned that limited availability of glycine, as substrate of glutathione synthase, would restrict flow through this enzyme and might thus result in increased urinary excretion of pyroglutamic acid. We have measured the urinary excretion of pyroglutamic acid in non-pregnant and pregnant women in the 1st, 2nd and 3rd trimesters.

	<i>n</i>	Pyroglutamic acid/creatinine ( $\mu\text{mol}/\text{mmol}$ )	
		Median	Range
Non-pregnant	11	10	2-30
1st Trimester	5	47	28-62
2nd Trimester	12	96	21-680
3rd Trimester	12	212	12-1210

There was a relatively small diurnal variation in pyroglutamic acid/creatinine and a single urine gave a representative value. As pregnancy advanced the pyroglutamic acid excretion rose progressively as predicted, and by the 3rd trimester was over twenty times greater than in the non-pregnant woman.

As only a portion of the pyroglutamic acid produced is normally excreted in the urine, it is possible that an increased excretion reflects a change in the metabolism of pyroglutamic acid unrelated to glycine metabolism. However, we have shown in normal adults that acute depletion of the available glycine pool with oral benzoic acid can give rise to pyroglutamic aciduria. We have found that excretion increases in four different metabolic states in which the demand for glycine is enhanced. These results provide support for the postulate that glycine acts as a semi-essential amino acid, and suggests that the availability of glycine may be limited as pregnancy advances. The excretion of pyroglutamic acid may provide a simple non-invasive index of glycine sufficiency.

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**Glucose metabolism in shorn and unshorn pregnant sheep.** By M. E.

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The shearing of ewes 8 weeks before lambing results in an increased plasma glucose concentration which is most apparent during the final 2 weeks of pregnancy (Symonds *et al.* 1985). It has been proposed that this response may result in a higher lamb birth weight due to an increased supply of glucose to the fetus. Shearing may induce changes in glucose metabolism either by stimulating hepatic gluconeogenesis or by inhibiting glucose utilization by hind-limb tissues. The aim of the present study was to quantify the effects of shearing on the supply and utilization of glucose by maternal tissues.

Five pairs of unshorn (US) and shorn (S) (at 8 weeks before lambing, i.e. 95 d of pregnancy) twin- or triplet-bearing Bluefaced Leicester cross Swaledale ewes were paired with respect to body-weight (62–87 kg). All animals were housed at ambient temperature (–2 to 13.5°) and given, twice daily at 08.30 and 18.00 hours, a diet of barley concentrate and ammonia-treated straw. Catheters were implanted into the carotid artery of all sheep and 8 d later into the femoral vein (119–139 d of pregnancy). The next day [U-<sup>14</sup>C]- and [6-<sup>3</sup>H]glucose were infused for a 5 h period starting after the morning feed, followed 4 d later by a 5 h infusion of NaH<sup>14</sup>CO<sub>3</sub>. Four sets of blood samples were taken during the final hour of the isotope infusion. Hind-limb blood flow rates were measured using tritiated water.

	Plasma glucose (mM)		Glucose ILR (mmol/min)		Glucose oxidation (mmol/min)		CO <sub>2</sub> ILR (mmol/min)		Glucose uptake (mmol/min per kg hind-limb tissue)		Plasma insulin (ng/ml)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
S	2.80	0.20	0.75*	0.03	0.55*	0.05	18.1*	2.1	0.030	0.010	0.30*	0.02
US	2.64	0.14	0.63	0.04	0.42	0.04	15.4	1.1	0.019	0.004	0.40	0.03

Mean value significantly different from US value (paired *t* test): \**P* < 0.05.

Shearing resulted in a significant increase in the rate of irreversible loss (ILR) of glucose but had no significant effect on glucose concentration or utilization by hind-limb tissues. There was also a greater CO<sub>2</sub> ILR in the S group plus a higher rate of whole body glucose oxidation.

It is concluded that shearing stimulates gluconeogenesis. Shearing was also associated with an increase in glucose oxidation despite lower plasma insulin concentrations which suggest that there was a change in tissue sensitivity to insulin in the S animals. These results indicate that there was an increased supply of glucose to maternal tissues of S sheep but no change in the utilization of glucose by hind-limb tissues.

M.E.S. acknowledges the support of a MAFF studentship.

Symonds, M. E., Bryant, M. J. & Lomax, M. A. (1985). *Proceedings of the Nutrition Society* **44**, 136A.

**Maternofetal iron transfer in the riboflavin-deficient rat.** By HILARY J. POWERS, *MRC Dunn Nutrition Unit, Cambridge CB4 1XJ*

During gestation, iron is diverted from maternal tissues as a consequence of fetal requirements but the factors controlling this transfer are not well understood. Riboflavin may be important in this process as it is believed to be involved in the mobilization of Fe from tissue ferritin (Sirivech *et al.* 1977; Powers *et al.* 1983). An experiment was set up to test this possibility.

Riboflavin-deficient ( $B_2^-$ ) dams and controls ( $B_2^+$ ), weight-matched until mid-pregnancy, were injected with a tracer dose of  $^{59}\text{Fe}$  on days 17 or 20 of gestation. Dams were killed 24 h later and the distribution of  $^{59}\text{Fe}$  between maternal liver, placenta and fetuses were measured. Ferritin Fe mobilizing activity by placental mitochondria was also measured. Maternal hepatic ferritin Fe and circulating Fe were also determined.

*Percentage of  $^{59}\text{Fe}$  dose reaching fetal tissues*

	Day 17-18				Day 20-21			
	Fetal mass (g)		$^{59}\text{Fe}$ (%)		Fetal mass (g)		$^{59}\text{Fe}$ (%)	
	$B_2^-$	$B_2^+$	$B_2^-$	$B_2^+$	$B_2^-$	$B_2^+$	$B_2^-$	$B_2^+$
Mean	2.11 <sup>***</sup>	6.25	0.75 <sup>***</sup>	2.22	17.42 <sup>**†</sup>	31.24 <sup>†</sup>	9.95 <sup>*†</sup>	17.20 <sup>†</sup>
SEM	0.77	0.26	0.17	0.13	2.74	1.52	1.00	1.70
n	5	7			4	7		

Mean values were significantly different from  $B_2^+$  values at the same time period (Student's *t* test): \* $P < 0.02$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

Mean values were significantly different from day 17-18 values (Student's *t* test): † $P < 0.001$ .

Half of the  $B_2^-$  dams showed almost complete fetal resorption; the remainder showed a significant reduction in fetal mass (Table). Riboflavin deficiency was associated with an altered distribution of  $^{59}\text{Fe}$  to the tissues (percentage of dose), notably with a reduction in the percentage reaching the fetuses (Table). When  $^{59}\text{Fe}$  content of tissues was expressed as counts/min per g, any previously observed differences between  $B_2^-$  and  $B_2^+$  animals disappeared. Hepatic ferritin Fe was significantly higher at day 18 in  $B_2^-$  animals (103.6 (SEM 12.4)  $\mu\text{g/g}$ ) than in controls (63.4 (SEM 9.2)  $\mu\text{g/g}$ ) ( $P < 0.02$ ) and, in contrast with the controls, had not fallen significantly by day 21 of gestation. Plasma Fe concentrations were substantially (although not significantly) higher in the  $B_2^-$  animals (19.9 (SEM 3.6)  $\mu\text{mol/l}$ ) compared with the controls (12.0 (SEM 1.8)  $\mu\text{mol/l}$ ) at day 18 and, unlike the controls, did not fall significantly over the subsequent 3 d. Finally, ferritin Fe mobilizing activity was observed in placental mitochondrial preparations but was not significantly influenced by either riboflavin status or stage of pregnancy.

There may be a causative association between the low hepatic Fe depletion and poor fetal weight gain in  $B_2^-$  animals or these may be independent effects of riboflavin deficiency.

Powers, H. J., Bates, C. J. & Duerden, J. M. (1983). *International Journal of Vitamin Nutrition Research* 53, 371-376.

Sirivech, S., Driskell, J. & Frieden, E. (1977). *Journal of Nutrition* 107, 739-745.

**The effect of conservation method and frequency of feeding on rumen microbial activity.** By E. R. PRATES, L. R. S. THIAGO, M. GILL and M. K. THEODOROU, *Animal and Grassland Research Institute, Hurley, Maidenhead, Berks SL6 5LR*

The research in this and the following two abstracts was designed to look at digestion in and outflow from the rumen in relation to the voluntary intake of hay and silage.

Perennial ryegrass (*Lolium perenne*) was cut on 8 June; half the crop was conserved as silage and half as hay. These diets were offered, either once or eight times daily, at 20 g dry matter (DM)/kg to twelve Friesian steers (initial live weight 127 kg) fitted with rumen cannulas (85 mm), and electrodes on the dorsal surface of the rumen for recording myoelectric activity.

Rates of digestion *in situ* were estimated from the disappearance of DM from dacron bags suspended in the rumen for 0, 1, 2, 3, 6, 9, 12, 48, 72 and 96 h using the equation of McDonald (1981). Feeding frequency had no effect on any of the indices measured (Table 1).

Table 1. *DM disappearance from material incubated in dacron bags*

Feeding frequency (/d)	Hay (H)		Silage (S)		Significance H v. S
	Once	Eight times	Once	Eight times	
Instantly degraded (%)	26.23	25.93	39.02	41.02	$P < 0.001$
Rate of disappearance (/h)	0.058	0.057	0.061	0.073	$P < 0.05$
Initial lag (h)	3.14	2.74	0.27	1.73	$P < 0.05$

Rumen microbial activity was assessed by measuring gas production *in vitro* using samples (two calves per treatment) obtained at specific times after feeding. There were no significant changes in gas production with time or diet for the animals fed eight times daily (13.15 ml/g DM on hay and 13.25 ml/g DM on silage). For the once-daily fed steers, gas production varied with period after feeding (Table 2) but not with diet.

Table 2. *Rate of gas production in vitro (ml/g DM)*

Period after feeding (h) . . .	0.5	1.5	6.0	11.0	16.0	18.5	23.5	SE of mean
Hay	15.5	17.1	13.6	13.2	13.1	11.0	9.4	1.07
Silage	20.6	16.5	13.2	10.5	9.5	10.7	12.1	1.26

The results show that while there appear to be changes in microbial activity over 24 h in animals fed once daily, these differences were not apparent from values obtained using dacron bags.

Financial help from CNPq Brazil is gratefully acknowledged (ERP).

McDonald, I. (1981). *Journal of Agricultural Science, Cambridge* 90, 251-252.

**Effect of conservation method and frequency of feeding on forestomach motility.** By J. W. SISSONS, M. GILL and L. R. S. THIAGO, *Animal and Grassland Research Institute, Hurley, Maidenhead, Berks SL6 5LR*

Earlier studies in growing steers adapted to diets varying in proportions of grass hay and concentrates showed a positive relation between the turnover rate of rumen fluid and frequency of reticular contractions as indicated by the occurrence of myoelectric spike bursts on the forestomach wall (Sissons *et al.* 1984). The present study was undertaken to examine rumen motor function, and consequently digesta passage, as affected by the nature of roughage and frequency of feeding.

In experiments described previously by Prates *et al.* (1986), recordings of myoelectric activity were made from the dorsal rumen of steers which received a diet of either hay or silage. The daily allowance was given either all at one time or divided into eight equal feeds/d. Myoelectric activity was recorded from two animals for each treatment using a Grass model 7 polygraph set to measure fast AC potentials in the range 10–35 Hz. Filtered signals were quantified using a summing integrator.

Recordings from steers fed eight times/d showed that when animals were not eating, frequencies of spike activity varied between 60 and 70 bursts/h, whereas for a 20 min period of ingestion immediately after offering silage or hay the frequency increased to a rate of between 80 and 120 bursts/h. Results of total activity/unit time also showed a uniform pattern throughout the 24 h recording period, except for variation in response to feeding which reflected the increase in contractile frequency.

Steers fed once a day showed elevations of spike burst frequency and total activity during eating, similar to those seen with animals fed eight times/d. However, subsequent patterns of total activity varied according to the nature of the roughage. For animals given hay there was a gradual increase in myoelectric activity during a period beginning at 7 h and reaching a maximum by about 13 h after feeding. Thereafter there was a small decline, but values only returned to basal levels by about 1 h before the next feed. A similar trend was observed in steers given silage once/d, but the period of maximal activity was less pronounced compared with measurements in hay-fed animals.

These results indicate that changes in contractile activity of the forestomach can be induced by alterations in the frequency of feeding and may be further influenced by the nature of conserved forage used in the diet. From earlier studies (Sissons *et al.* 1984) it may be predicted that the wide variation in ruminal motor responses found here in steers fed once rather than eight times daily, would probably give rise to a relatively uneven pattern of digesta passage from the forestomach, especially in animals given hay.

Prates, E. R., Thiago, L. R. S., Gill, M. & Theodorou, M. K. (1986). *Proceedings of the Nutrition Society* **45**, 95A.

Sissons, J. W., Thurston, S. M. & Smith, R. H. (1984). *Canadian Journal of Animal Science* **64**, 70–71.

**The effect of conservation method and frequency of feeding on the removal of digesta from the rumen.** By L. R. S. THIAGO and M. GILL, *Animal and Grassland Research Institute, Hurley, Maidenhead, Berks SL6 5LR*

Work by Aitchison (1985) with sheep given hay once daily showed marked differences in rumen fill with time after feeding. The work described here was undertaken to study the patterns of rumen fill in cattle offered hay and silage once or eight times/d.

The animals and diets used were described by Prates *et al.* (1986). Rumen fill was estimated by complete manual emptying of the rumen at specific times after feeding (09.00 hours for once daily fed steers), allowing 2 d between successive emptyings. The silage had a pH of 3.8, toluene dry matter (DM) of 212 g/kg DM and a total N of 29 g/kg DM. The hay had a DM of 822 g/kg.

For steers fed eight times/d, DM in the rumen did not vary significantly with time but was significantly higher for hay than silage ( $P < 0.05$ ). For animals fed once daily, the weight of digesta DM in the rumen of silage-fed steers increased more slowly and reached a lower maximum than did that of hay-fed steers (Fig. 1).

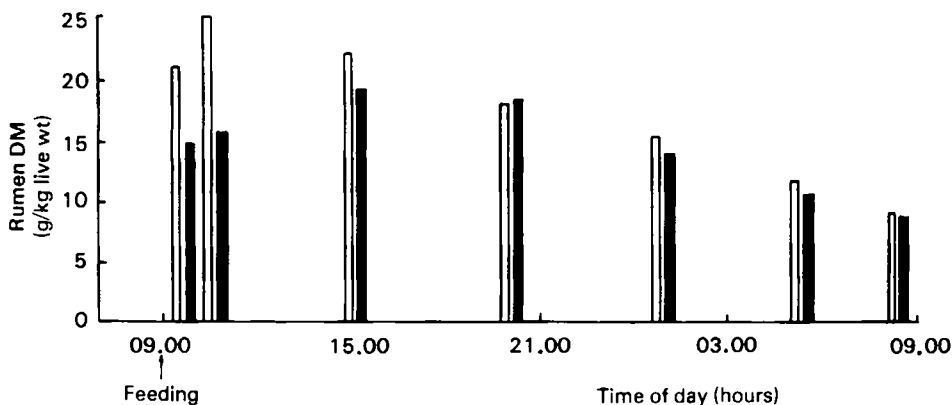


Fig. 1. Mean rumen DM pool size of hay- (□) and silage- (■) fed steers.

This slower increase partly reflects the longer time taken to eat the daily ration of silage than of hay, but may also be a result of the more rapid initial digestion of the silage (Prates *et al.* 1986). From 12 to 24 h after feeding, the amount of digesta in the rumen appeared to be similar for both diets. Thus the main difference between the two forages was in the first 6 h after feeding.

Aitchison, E. (1985). A study of the removal of fibre from the rumen and voluntary intake of sheep eating hay diets, PhD Thesis, Reading University.

Prates, E. R., Thiago, L. R. S., Gill, M. & Theodorou, M. K. (1986). *Proceedings of the Nutrition Society* 45, 95A.



**Effect of ambient temperature and urea supplementation on the intake of fibrous diets by draught ruminants.** By N. A. C. WATSON\*, R. ANNE PEARSON and R. F. ARCHIBALD, *Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian EH25 9RG*

Draught animals often have to survive on fibrous crop residues. The nutritive value of such feeds may be improved by treating with alkali or urea, or both, as an alternative to supplementation with more nutritious although often scarce and expensive concentrate feed. The responses of two species of draught animals to poor quality roughage treated in this way has been studied.

Four adult swamp buffalo and four adult Brahman cattle, maintained in climate chambers, were provided *ad lib.* with a pelleted alkali-treated straw ration supplemented with sulphur, minerals and vitamins and one of three levels of urea: 0 (TS), 12 (TSU<sub>1</sub>) or 24 (TSU<sub>2</sub>) g/kg. The animals were given each diet when housed at 32° and, in addition, were given the TS diet at 22°. All animals received all treatments in periods lasting 20 d with measurements made during the last 7 d.

Breed	Buffalo				Brahman				SE of difference	
	22 TS	32 TS	32 TSU <sub>1</sub>	32 TSU <sub>2</sub>	22 TS	32 TS	32 TSU <sub>1</sub>	32 TSU <sub>2</sub>	Between species	Within species
Temperature (°)										
Diet										
Respiration rate (breaths/min)	15	18	17	19	19	25	24	25	1.53	2.70
Plasma 3,5,3'- triiodothyronine	1.24	0.76	1.08	1.16	1.33	0.61	1.13	0.87	0.21	0.17
DM intake (g/kg live wt <sup>0.75</sup> per d)	63	61	90	91	68	64	107	105	12.82	4.56
Apparent digestibility										
DM (%)	52	54	60	61	50	53	59	60	3.67	2.81
N (%)	27	29	58	64	27	32	58	64	3.61	4.02
Live-wt gain (kg/d)	-1.46	-1.44	1.19	1.59	-1.52	-0.33	0.15	0.93	0.66	0.81

Significant ( $P < 0.01$ ) reductions in plasma 3,5,3'-triiodothyronine concentrations were observed in all animals at the higher temperature. The Brahmans showed significantly ( $P < 0.01$ ) higher respiration rates than the buffalo at the higher temperature. Despite these physiological changes in response to temperature, there were no significant differences, due to temperature, in voluntary food intake or digestibility of dry matter (DM) and nitrogen in either species.

Supplementation of the alkali-treated straw with urea (12 g/kg) resulted in a significant ( $P < 0.001$ ) increase in voluntary food intake by both species and significant increases in digestibility of DM ( $P < 0.05$ ) and N ( $P < 0.001$ ). No significant differences between species in their ability to consume or digest the straw-based diets were observed.

An increase in urea supplementation from 12 to 24 g/kg had little further beneficial effect on food intake or digestibility of the straw diet.

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**Phosphate fluxes across the rumen wall mucosa of sheep in vitro.** By G. BREVES, G. GAEBEL, H. MARTENS and H. HOELLER, *Department of Physiology, School of Veterinary Medicine, Bischofsholer Damm 15, D-3000 Hannover 1, Federal Republic of Germany*

Although the forestomach is not considered to be the major site of phosphate (P) absorption in sheep (Scarlsbrick & Ewer, 1951; Parthasarathy *et al.* 1952) it was recently found that P net absorption in vivo from the rumen increased linearly when P concentrations increased from 0 to 15 mmol/l (Breves & Hoeller, 1986).

In vitro experiments on the mechanisms of ruminal P transport were performed in Ussing chambers under short-circuit conditions with fifty ruminal mucous membranes from seven sheep. The tissues were prepared within 2–3 min after slaughter and then incubated in isotonic buffer solution, pH 7.3, consisting of NaCl, KCl, NaHCO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, CaCl<sub>2</sub>·2H<sub>2</sub>O, MgCl<sub>2</sub>·6H<sub>2</sub>O, Na acetate·3H<sub>2</sub>O, Na propionate, butyric acid, NaOH and glucose and gassed with carbogen. The P concentration was 3 mmol/l. P fluxes from mucosa to serosa ( $J_{ms}^P$ ) and from serosa to mucosa ( $J_{sm}^P$ ) were measured by adding 8  $\mu$ Ci <sup>32</sup>P either to the mucosal or serosal sides of each chamber. Up to nine flux periods of 30 min each were done in each chamber; the last two periods were performed after addition of ouabain (0.1 mmol/l) to the serosal side.

The mean unidirectional fluxes of P and the electrical indices of the tissues (conductance (GT), short-circuit (I)) are shown in the table.

	$J_{ms}^P$		$J_{sm}^P$	
	Mean	SD	Mean	SD
Without ouabain				
Flux (nmol/h per cm <sup>2</sup> )	5.9*	1.6	4.5	1.1
GT (ms/cm <sup>2</sup> )	2.77	0.70	2.51	0.72
I ( $\mu$ eq/h per cm <sup>2</sup> )	0.68	0.35	0.78	0.41
With ouabain				
Flux (nmol/h per cm <sup>2</sup> )	7.9	2.7	7.9	3.0
GT (ms/cm <sup>2</sup> )	3.36	0.98	3.13	1.10
I ( $\mu$ eq/h per cm <sup>2</sup> )	-0.34	0.50	-0.24	0.51

\* $P < 0.05$ .

The flux from mucosa to serosa was slightly higher ( $P < 0.05$ ) than  $J_{sm}^P$ . Since this difference was abolished by ouabain the results indicate that P net transport to the serosal side may depend on Na, K-ATPase activity.

The investigations were supported by a grant from the German Research Foundation (DFG).

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**Hypoglycaemia and luteinizing hormone release in the ewe.** ALISON D. CRUMP and R. G. RODWAY, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

It has been suggested that the reduced fertility seen in cattle and sheep at times of nutritional or metabolic stress may be caused, at least in part, by the low blood glucose concentrations of such animals (McClure, 1965). In the present experiment we have studied the release of luteinizing hormone (LH) in anoestrous ewes made hypoglycaemic by the infusion of insulin.

Eight mature seasonally anoestrous ewes were used in a total of twenty-eight trials. Saline (9 g sodium chloride/l) ( $n$  17) or insulin ( $n$  11) was infused into the jugular vein for 48-h periods. The insulin infusion rates was increased approximately every 8 h (initial rate 123 mU/min; final rate 314 mU/min) in order to maintain a constant level of hypoglycaemia. Mean blood glucose concentrations over the 48-h period were 4.43 (SEM 0.07) mM (control) and 2.44 (SEM 0.07) mM (insulin). In order to induce a surge release of LH similar in magnitude to the natural preovulatory surge, oestradiol (1  $\mu$ g/kg) was given subcutaneously either 8 or 16 h after the start of the infusion. Blood samples were collected every 30 min via jugular catheters during the expected time of the LH peak. Neither the time from oestradiol injection to the LH peak (control 15.9 (SEM 0.6) h; insulin 15.2 (SEM 0.5) h) nor the mean peak height (control 62.9 (SEM 9.0) ng/ml; insulin 67.8 (SEM 9.6) ng/ml) was affected by insulin infusion. However, the number of animals not giving an LH peak in response to oestradiol was significantly greater in the hypoglycaemic group (control 1/17; insulin 5/11;  $P < 0.001$ ). Plasma concentrations of non-esterified fatty acids (control 238 (SEM 23)  $\mu$ mol/l; insulin 403 (SEM 49)  $\mu$ mol/l) and cortisol (control 20.9 (SEM 3.0) mM; insulin 69.1 (SEM 8.4) mM) were both increased by insulin infusion, indicating that the degree of hypoglycaemia achieved was having considerable metabolic effects. The increase in non-esterified fatty acid concentration was similar in magnitude to that caused by fairly severe undernutrition for 50 d (Crump *et al.* 1985).

It is concluded that hypoglycaemia, although not affecting the timing of the amount of LH released, did significantly reduce the number of animals giving an LH peak, and therefore may be a contributory factor in the anovulatory condition seen in severe energy restriction.

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**Adipose tissue cellularity and site-specific differences in adipocyte volume in genetically obese (*ob/ob*) mice.** By N. J. PHILIPPS, *Department of Zoology, University of Bristol, Bristol BS 1UG* and C. M. POND, *Department of Biology, The Open University, Milton Keynes MK7 6AA*

Genetically obese (*ob/ob*) mice are larger and fatter, and show several metabolic differences from similarly maintained non-mutant litter mates (Enser, 1972). Using established dissection and adipocyte sizing techniques (Pond *et al.* 1984), we measured the gross mass and the diameter of about forty adipocytes of the nine largest adipose depots in normal and mutant female mice aged 20 weeks, fed *ad lib* on normal laboratory chow at 20°. The site-specific mean adipocyte volume and the cellularity of each depot were calculated using standard formulae (Goldrick, 1967) and taking the density of adipose tissue as 0.9. The mean number of adipocytes per kg body mass in the mutant mice was  $2.18 \times 10^8$ , which was significantly different ( $P < 0.001$ ) from the mean value of  $1.40 \times 10^8$  in the normal mice. Although the total adipocyte complement of the mutant mice was 14% greater than that predicted from the allometric relation between adipose tissue cellularity and body mass (Pond & Mattacks, 1985), it was within the range of inter-individual differences in adipocyte complement found in apparently normal wild mammals. Most of the additional adipocytes were in the mesenteric, shoulder, side groin and tail depots. Mean volumes of adipocytes in all depots studied were greater in the mutants than in the controls but, in both strains, adipocytes in the 'under trapezius muscles' depot were smaller, and those in the two inguinal depots larger, than those in the mesentery and in the shoulder and fore-limb depots. This pattern of site-specific differences in adipocyte volume is similar to that described in many other mammalian species (Pond, 1986).

	Fore-limb		Shoulder		Inguinal		Tail	Mesentery	Under trapezius muscles
	Anterior	Posterior	Anterior	Medial	Lateral	Medial			
	Normal mice ( <i>n</i> 17)								
Mean (nl)	0.141	0.299	0.201	0.217	0.452	0.483	0.544	0.432	0.046
SD (nl)	0.013	0.021	0.012	0.021	0.032	0.030	0.034	0.030	0.004
	<i>ob/ob</i> mutant mice ( <i>n</i> 9)								
Mean (nl)	0.041	1.500	1.331	1.018	2.107	2.094	1.575	2.010	0.372
SD (nl)	0.115	0.111	0.104	0.102	0.195	0.204	0.077	0.104	0.034

We conclude that obesity in *ob/ob* mice is due mainly to adipocyte enlargement at all depots studied and that the typical mammalian pattern of site-specific differences in adipocyte volume is present in this mutant.

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**Fatness of laying hens and their response to reduced energy intakes.** By S. P. ROSE and A. P. LEAN, *The North of Scotland College of Agriculture, 581 King Street, Aberdeen AB9 1UD*

An experiment was performed to examine the response of laying hens differing in fatness to dietary regimens which reduced their energy intakes. Abdominal fatness caliper measurements (Rose & Michie, 1983) were taken on 1240 Isa Brown hens at 44 weeks of age. The 432 birds with the highest and lowest measurements were allocated to 'fat' and 'lean' groups respectively. Dissection of a sample of these birds showed that the caliper measurements were significantly correlated ( $r = 0.688$ ) to the weight of the abdominal fat as a proportion of live weight. The birds were re-caged into twenty-four experimental units and given one of three dietary treatments for a 24 week period: (i) a conventional layer's food (11.6 MJ metabolizable energy (ME)/kg, 170 g crude protein (nitrogen  $\times$  6.25)/kg and 40 g calcium/kg) *ad lib.*; (ii) a low-energy food *ad lib.* in which the calculated nutrient composition differed only from the conventional food in ME (10.6 MJ ME/kg); (iii) the conventional food restricted to 133.5 g/bird per d.

The fat birds (6.6% abdominal fat) were 220 g heavier than the lean birds (4.2% abdominal fat) at the start of the experiment and this difference increased to 340 g in the *ad lib.*-fed birds by the end of the experiment. The proportion of abdominal fat increased in both the fat and lean birds given the conventional and low-energy foods *ad lib.* but remained approximately constant in the layers given the restricted quantity of the conventional food.

Fatness group	Diet	n	Egg numbers (no./100 bird days)	Egg weight (g)	Food intake (g/bird per d)	Calculated energy intake (MJ/bird per d)
Fat	Conventional	4	75.39	67.19	130.3	1.512
	Restricted	4	76.09	64.98	113.5	1.317
	Low energy	4	76.49	66.67	134.2	1.369
Lean	Conventional	4	76.62	67.20	125.1	1.451
	Restricted	4	74.17	66.79	113.2	1.313
	Low energy	4	77.13	67.27	131.4	1.393
	SED	4	1.445	0.515	2.11	0.024

The number of eggs laid and mean egg weight were similar for both the fat and lean birds given the conventional food *ad lib.* However, the fat birds given the restricted quantity of food had a reduced ( $P < 0.05$ ) egg weight, whereas the restricted lean birds had a similar ( $P > 0.05$ ) egg weight compared with the birds given food *ad lib.* The layers given the low-energy food also had a reduced energy intake compared with the birds given the conventional food, but egg numbers and weights were not altered ( $P > 0.05$ ).

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**Effects of parental age on fatty acid oxidation in vitro by the liver and heart of the chick embryo.** By R. C. NOBLE, J. H. SHAND and K. CONNOR, *Hannah Research Institute, Ayr KA6 5HL*, and D. BROWN, *West of Scotland Agricultural College, Ayr KA6 5HW*

Parental age of the broiler bird considerably affects embryo hatchability and chick survival (Shanawany, 1985). With the need to procure hatching eggs at earlier stages of the hen's reproductive life in order to increase chick yield in the broiler industry, lowered hatchability is a problem. Lipid metabolism is intense during the last week of embryonic development, and investigations have shown that reduced hatchability of eggs from very young parents is associated with alterations in yolk lipid transfer and in the lipid composition of embryonic tissues (Noble *et al.* 1986). The possibility of associated disturbances to oxidation catabolism of the yolk lipid has now been investigated.

Liver and heart tissue were excised from chick embryos of eggs from broiler-breeder parent birds during the first 3 weeks of lay (23–25 weeks old) and during maximum lay (33 weeks old). Following homogenization in Krebs-Ringer buffer, the rate of fatty acid oxidation was determined by the addition of 1-[<sup>14</sup>C]palmitic acid and measurement of <sup>14</sup>CO<sub>2</sub> during incubation for 2 h at 37°. Apart from embryos from 33-week-old parents, the rates of oxidation by the livers at day 15 of development were higher ( $P < 0.001$ ) than those at day 19 (see Table). However, the rates of oxidation in the livers of 15-d-old embryos from 23- and 24-week-old parents were much greater ( $P < 0.001$ ) than those from 25- and 33-week-old parents. The rates of oxidation in heart tissue at days 15 and 19 of development showed no such pattern in their oxidation rates, other than in embryos from 24-week-old parents the rates of oxidation were in general similar to each other.

*Oxidation of 1-[<sup>14</sup>C]palmitic acid (nmol/min per mg protein) in the embryo livers and hearts (n 4)*

Parental age (weeks)...	23		24		25		33	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver:								
day 15	0.91	0.05	3.76	0.19	0.47	0.03	0.40	0.01
day 19	0.20	0.01	0.16	0.01	0.21	0.02	0.41	0.03
Heart:								
day 15	1.20	0.07	1.18	0.04	1.14	0.06	0.96	0.07
day 19	1.49	0.08	0.63	0.03	0.91	0.04	1.28	0.03

As well as distinct changes in yolk lipid uptake and tissue lipid composition, the higher mortality of the chick embryos from very young parents appears also to be associated with changes in hepatic oxidation of the assimilated lipid. Although evidence exists that this may be associated with a slower rate of embryonic development, the extent of the changes suggests a more specific metabolic disorder.

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**The viscosity of viscous polysaccharides may change after ingestion.** By C. A. EDWARDS<sup>1</sup>, P. A. DAVISON<sup>1</sup>, J. TOMLIN<sup>1</sup>, N. A. BLACKBURN<sup>2</sup>, L. CRAIGEN<sup>1</sup>, K. SUGDEN<sup>2</sup>, H. JARJIS<sup>1</sup>, P. G. HESSEL<sup>2</sup>, C. PARK<sup>1</sup>, J. G. LLOYD-JONES<sup>2</sup>, I. R. FLOCKHART<sup>2</sup>, I. T. JOHNSON<sup>3</sup> and N. W. READ<sup>1</sup>, <sup>1</sup>*Clinical Research Unit, Royal Hallamshire Hospital, Sheffield S10 2JF*, <sup>2</sup>*Reckitt and Colman Pharmaceuticals, Dansom Lane, Hull* and <sup>3</sup>*Food Research Institute, Colney Lane, Norwich*

The relative abilities of guar gum (G, molecular weight (MW) 220 000; T. M. Duche and Sons Ltd), xanthan (X, MW 310 000; Ketrol F grade, Kelco), Locust bean gum (LBG, MW >10<sup>6</sup>; T. M. Duche and Sons Ltd) and a 1:1 w/w mixture of X/LBG to inhibit glucose movement and absorption in vitro and in vivo were assessed. X/LBG was the most viscous at all shear rates (1.92–384/s; Brookfield cone and plate viscometer) and all concentrations (1–10 g/l). The movement of radiolabelled glucose across a three-compartment cell was inhibited by all the gums (1 g/l,  $P < 0.05$ ) but X/LBG was the most effective ( $P < 0.05$ ). X/LBG also reduced glucose absorption from tied intestinal loops from rats more efficiently than the other gums, but when it was incorporated into a 250 ml orange glucose (50 g) drink (10 g gum/l) given to healthy human volunteers, it was no more effective than the other gums at reducing postprandial hyperglycaemia although it alone delayed gastric emptying.

To investigate this apparent discrepancy, the viscosities of the polysaccharides (10 g/l) were measured before and after acidification in vitro to pH 2 with a solution of 100 mM-hydrochloric acid, 54 mM-sodium chloride and again after reneutralization with a solution of 120 mM-sodium bicarbonate, 5 mM-potassium chloride, 30 mM-NaCl to mimic events occurring to the gums after ingestion. Polysaccharide solution diluted with the same volumes of 154 mM-NaCl acted as a control. This procedure altered the viscosity (at a shear rate of 11.2/s) of most of the polysaccharides (Table). X/LBG was affected most; its final viscosity was not much higher than that of the other polysaccharides which may explain its disappointing effect on postprandial hyperglycaemia.

*Viscosities† (mPa.s) of polysaccharide solutions (n 5)*

Polysaccharide treatment and concentration (g/l)	Initial 10		Acidified 8.9		Control 8.9		Neutralized 6.8		Control 6.8	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
	G	2396	51	1939*	78	1714	160	1209	51	1227
X	1128	28	1558	131	1544	24	1384**	29	1261	22
LBG	2121	73	1579	76	1602	53	939	54	984	36
X/LBG	9922	524	1294***	142	4474	595	2138***	68	1391	78

Significantly different from the control value at the same concentration of polysaccharide:  
\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

†Viscosity measured with Contraves rotatory viscometer.

Thus in vitro viscosity measurements may be misleading when used to predict the action of ingested polysaccharides.

**Degradation of guar gum by faecal bacteria.** By J. TOMLIN<sup>1</sup>, C. A. EDWARDS<sup>2</sup>, B. I. DUERDEN<sup>2</sup> and N. W. READ<sup>1</sup>, <sup>1</sup>*Clinical Research Unit and* <sup>2</sup>*Department of Medical Microbiology, Royal Hallamshire Hospital, Sheffield S10 2JF*

Degradation of guar gum by faecal bacteria was investigated in vitro by studying the changes in viscosity and pH, and the production of hydrogen gas when the gum was incubated with faecal suspensions at 37° under anaerobic conditions. Incubation for 21 h reduced the viscosity of a solution of guar gum (5 g/l) from (mean (SE)) 173 (12.4) mPa.s (*n* 6) to 2.0 (0.0) mPa.s (*P*<0.01), and generated H<sub>2</sub> gas (6.38 (3.17) %) from cultures containing 0.5 g faeces. There was no change in these indices when guar gum was incubated with a suspension of heat-sterilized faeces (121°, 20 min), but a bacteria-free filtrate of the faecal suspension reduced the viscosity from 174.4 (35.2) mPa.s to 13.3 (3.2) mPa.s (*P*<0.01) without affecting the pH or generating H<sub>2</sub>. These results suggest that the viscosity of guar gum can be reduced by an extracellular enzyme present in the faecal filtrate but that intact bacteria are necessary for fermentation. To determine whether different organisms may collaborate to degrade guar gum, we incubated single strains of bacteria isolated from faeces with gum that had been treated with a bacteria-free filtrate of faeces and with untreated guar gum. Only a few strains of *Bacteroides* could ferment guar gum (two of six strains of *B.fragilis*, 3/6 *B.ovatus*, 1/3 *B.variabilis*, 1/2 *B.uniformis*). Two strains (1/6 *B.distasonis*, 1/6 *B.thetaiotaomicron*) were only able to ferment guar gum that had been pretreated with a bacteria-free filtrate of faeces. One strain (1/3 *B.variabilis*) reduced the viscosity of untreated guar gum but showed no signs of fermentation. This suggests that different species can collaborate to digest guar gum, but to a limited degree. Extracellular enzymes released by some bacterial strains make the gum available to additional strains. The large variations in the ability to ferment guar gum between strains of the same species support observations (Bayliss & Houston, 1984) that guar-gum fermenters do not fit into any of the established categories that are based on simple biochemical characteristics.

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