The Editors of the Proceedings of the Nutrition Society *accept no responsibility for the abstracts of papers read at the Society's meetings for original communications.*

PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATION

The Three Hundred and Sixty-fifth Meeting of the Nutrition Society (One Hundred and Forty-Second of the Scottish Group) was held in the Hugh Nesbit Building, Riccarton Campus, Heriot-Watt University, Edinburgh, on Thursday and Friday, 17/18 *September* 1981 *when the following papers were read:*

https://doi.org/10.1079/PNS19820014 Published online by Cambridge University Press

Effect of cobalt deficiency on phosphatidylcholine and phosphatidylethanolamine in sheep liver. By A. K. LOUGH, W. R. H. DUNCAN, C. R. A. EARL and LESLEY COUTTS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

As part of a study of effects associated with cobalt deficiency in sheep and lambs (Fell, 1981; Garton *et al.* 1981) an examination was made of the phospholipid composition of liver tissue from ewes fed for up to 18 months on a diet of hay, flaked maize, minerals and vitamin mix (0·05 mg Co/kg dry matter). Control animals received cobalt intraruminally. Determinations of plasma vitamin B_{12} and of urinary methylmalonic acid (MMA) (Duncan *et al.* 1981) were made at intervals. Samples of liver obtained at slaughter were stored at −20°; lipids extracted into chloroform–methanol were subjected to two-dimensional thin-layer chromatography and the phosphorus content of the constituent phospholipids was estimated (Rouser *et al.* 1976). Values given in the Table for B_{12} and MMA were obtained within 14 d of slaughter.

Molar ratio of hepatic phosphatidylethanolamine (PE) to phosphatidylcholine (PC), urinary MMA and plasma vitamin B_{12} in ewes

	Co-supplemented			Co-depleted					
Ewe No.	1	2	3	4	5	6	7	8	9
PE:PC	0·50	0·48	0·48	0·87	0·80	0·71	0·58	0·47	0·45
MMA (g/d)	0·11	0·06	0·05	3·00	1·10	1·54	0·11	0·90	0·08
B_{12} (ng/ml)	2·00	6·00	3·00	0·29	0·24	0·10	0·40	0·15	0·24

Molar proportions of phosphatidylethanolamine to phosphatidylcholine (PE:PC) were markedly increased in cobalt-depleted ewes (4–6) when the deficiency state was associated with the urinary excretion of more than 1 g MMA/d; in these ewes intake of feed decreased markedly at least 2 months before slaughter. In ewes 7–9 intake of feed was similar to that of the control animals as was the proportion of PE:PC, even though urinary excretion of MMA by ewe 8 was increased.

It seems likely that the increased hepatic PE:PC ratios are associated with a reduction in the enzymic methylation of PE to PC such as has been observed in the livers of B_{12}-deficient rats (Åkesson *et al.* 1978). Decreased production of PC can be attributed to reduced availability of the methyl donor, S-adenosylmethionine, the liver content of which is decreased in B_{12}-deficient sheep (Gawthorne & Smith, 1974).

It is hoped to explore some of the implications of these changes in tissue phospholipid composition in relation to membrane structure and function.

Åkesson, B., Fehling, C. & Jägerstaad, M. (1978). *Br. J. Nutr.* **40**, 521.
Duncan, W. R. H., Morrison, E. R. & Garton, G. A. (1981). *Br. J. Nutr.* **46**, 337.
Fell, B. F. (1981). *Phil. Trans. R. Soc. Lond.* B (In the Press).
Garton, G. A., Duncan, W. R. H. & Fell, B. F. (1981). In *Symposium: Trace Element Metabolism in Man and Animals*—4, Australia: Academy of Science **294**, 153.
Gawthorne, J. M. & Smith, R. M. (1974). *Biochem. J.* **142**, 119.
Rouser, G., Kritchevsky, G. & Yamamoto, A. (1976). In *Lipid Chromatographic Analysis* Vol. 3, 2nd ed. p. 713 [G. V. Marinetti, editor]. New York: Marcel Dekker Inc.

A study of the effect of zinc on iron absorption in man. By R. W. CROFTON, D. GVOZDANOVIC and P. J. AGGETT, *Departments of Gastroenterology and Biomedical Physics and Bio-Engineering, University of Aberdeen*

Studies in animal models suggest that interactions between iron and zinc may occur during their uptake and absorption by the gastrointestinal tract (Hamilton *et al.* 1978). In this crossover study the effect of Zn on Fe absorption has been investigated in eight healthy male volunteers. After an overnight fast each subject received either 47 mg (0·84 mmol) of elemental Fe as ferrous sulphate or 47 mg of Fe in combination with 22·5 mg (0·34 mmol) of elemental Zn as zinc sulphate (molar ratio Fe:Zn=2·47:1). In both mixtures the iron was labelled with 10 μCi ^{59}Fe. The mixtures were given at least 6 weeks apart.

Increments in total plasma Fe (PFe) and ^{59}Fe in the 6 h following ingestion of the mixtures were plotted against time and used as indices of Fe absorption. The biological retention of the ^{59}Fe was determined by whole body monitoring.

The plasma ^{59}Fe and PFe increments were reduced in seven of the eight patients when Fe and Zn were given simultaneously. The PFe increment (mean±SE) was 71·6±17·6 μmol/l per h after Fe alone and 54·7±12·5 μmol/l per h after Fe+Zn in the 6 h post dose. Similarly, Fe absorption as assessed by plasma ^{59}Fe was reduced from 285±74 μmol to 176±51 μmol. The whole body retention of ^{59}Fe was similar following both doses at 9·8 and 9·1% of the administered dose for Fe and Fe+Zn mixtures respectively. None of these differences were statistically significant ($P>0·05$). In a further study five healthy male volunteers were given solutions containing 23·5 mg elemental Fe alone and with 27·5 mg Zn (molar ratio 1:1) and 68·8 mg Zn (molar ratio 1:2·5). Fe absorption as indicated by PFe was reduced by the addition of Zn from 87·1±12·3 to 17·7±9·2 with the equimolar solutions and to 9·1±12·2 with the 1:2·5 Fe–Zn solution ($P<0·01$).

These initial results indicate that Zn may affect the bio-availability of inorganic Fe at the ratio and dosages used in this study. Such an interaction is important in planning nutritional mineral supplementation and merits further evaluation.

Hamilton, D. L., Bellamy, J. E. L., Valberg, J. D. & Valberg, L. S. (1978). *Can. J. Physiol. Pharmac.* **56**, 384.

Efficiency of progesterone withdrawal as a factor in low zinc-induced dystocia. By G. E. BUNCE, C. F. MILLS, G. WILSON and A. KLOPPER, *Rowett Research Institute, Aberdeen AB2 9SB and Department of Obstetrics and Gynaecology, University of Aberdeen Medical School, Aberdeen AB9 2ZB*

The female rat offered a low-zinc diet during pregnancy experiences delayed and prolonged parturition (Apgar, 1968) associated with a shock-like condition in the dam and an increased incidence of mortality of both dams and foetuses. Uteri from Zn-deficient rats have been shown to have a decreased capacity for conversion of arachidonic acid to 2-series prostaglandins (Cunnane, 1981*a*) and severely depressed spontaneous contractility (Cunnane, 1981*b*). Since luteolysis and progesterone (Pr) withdrawal are necessary for the conversion of the quiescent uterus to an active state, the competency of Zn-deficient rats to accomplish this process was investigated.

Second parity Hooded Lister rats (Rowett strain) was maintained from day 7 of gestation on a semi-synthetic diet containing either <1 or 40 μg Zn/kg. Both pair-fed (PFC) and *ad lib.*-fed (ALC) controls were used. Rats were killed by exsanguination under pentobarbital anesthesia on days 19, 20, 21, 22 or 23 of pregnancy (delivery normally beings after 22 d). Heparinized plasma was analysed for Pr by radio-immunoassay. Ovaries were homogenized in 0·25 M-sucrose, centrifuged at 85000 *g* for 30 min and the supernatant fluid was analysed for activity of 20 α-hydroxysteroid dehydrogenase (20 α-OHSDH).

Dietary treatment influenced neither the mean nor the range of plasma Pr on days 19, 20 or 21. Between day 21 and 22, mean Pr in all groups fell from approximately 110 to 40 nmol/l but, whereas eight out of twelve PFC rats and eight out of ten ALC rats were at or below 30 nmol/l, only four out of fifteen Zn deficient rats were in this range. Wiest (1970) has proposed that parturition in the rat cannot begin until uterine Pr falls below 2 μg/100 g tissue. A similar threshold for plasma Pr has not been established, but examination of the literature suggests that values of <30 nmol/l are closely correlated with release of the Pr block.

Progesterone withdrawal in the rat results from a combination of two events: firstly, a decrease in the steroidogenic activity of the ovary and secondly, the induction of ovarian 20 α-OHSDH which converts the residual pregnenelone precursor to inactive 20 α-hydroxy Pr. Zn deficiency caused the rate of appearance of 20 α-OHSDH in the ovary to be inhibited by approximately 50 and 66% on days 22 and 23 of pregnancy. Observations that delivery was either imminent or concluded by 10.00 hours on day 23 in five out of seven ALC rats and six out of eight PFC rats compared to only three out of seven Zn-deficient rats are consistent with the possibility that delayed or inhibited induction of 20 α-OHSDH may be relevant to the above effects of Zn deficiency.

The results suggest that the dystocia of Zn deficiency may in part be explained by an inability to conclude luteolysis in a rapid and efficient manner.

Apgar, J. (1968). *Am. J. Physiol.* **215**, 160.
Cunnane, S. (1981*a*). *Proc. Nutr. Soc.* **40**, 78A.
Cunnane, S. (1981*b*). *Proc. Nutr. Soc.* **40**, 80A.
Wiest, W. (1970). *Endocrinology* **87**, 43.

Influence of liver copper concentration on plasma Cu depletion and repletion in the rat. By J. PRICE, J. K. CHESTERS and M. WILL, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The feasibility of assessing the relative availabilities of different forms of dietary copper from the rate of increase in plasma Cu concentration in the hypocupraemic rat given Cu orally by gavage is being investigated. In these studies, large differences in the rate of change in plasma Cu concentration have been observed within groups of animals during both the preliminary depletion phase and during repletion with Cu in the form of copper sulphate solution. The possibility that these differences may have arisen as a result of mobilization of Cu, to a varying degree, from the liver into the plasma was therefore examined.

Plasma Cu concentration was depleted in twenty-four Hooded Lister male rats weighing 120 g by feeding the animals on a semi-synthetic diet containing 0·5 mg Cu/kg. After a 7 d depletion period, ten rats with plasma Cu concentrations ranging from 0·10 to 0·70 μg/ml (Group A) were killed and the livers retained for Cu determination. The remaining rats, which were maintained on the low Cu diet, were allocated at random to two groups and given 20 (Group B) or 35 (Group C) μg Cu by gavage in 1 ml of aqueous copper sulphate solution on four consecutive days; the animals were killed 24 h after the final dose of Cu. Blood samples were obtained from the tail during the depletion and repletion phases and plasma Cu concentrations determined by atomic absorption spectrophotometry using a graphite furnace technique.

The group mean plasma Cu concentrations (±SE) before depletion were 0·77±0·03, 0·76±0·02 and 0·76±0·03 for Groups A, B and C respectively. After 7 d depletion the concentration of Cu in plasma (CuP, μg/ml) in rats from Group A was lowest in those animals with the lowest liver Cu concentrations (CuL_1, μg/g DM). This relationship could be described by the equation: $CuP = 0{\cdot}12\ CuL_1 - 1{\cdot}04$, RSD 0·11 ($P<0{\cdot}001$). There was also a significant relationship between the increase in plasma Cu concentration (△CuP, μg/ml) and liver Cu concentration (CuL_2) in animals from Group B 24 h after the final dose: $\triangle CuP = 0{\cdot}16\ CuL_2 - 1{\cdot}31$, RSD=0·26 ($P<0{\cdot}01$). At the higher dose rate (Group C) no significant effect of liver Cu on plasma Cu increase was observed.

https://doi.org/10.1079/PNS19820014 Published online by Cambridge University Press

Selenium status in paediatric health and disease states. By KATHRYN WARD, JUDY ANDERSON, J. R. ARTHUR and P. J. AGGETT, *Department of Child Health, Aberdeen University Medical School and Rowett Research Institute, Bucksburn*

Long-term selenium status was estimated in children from North-East Scotland using whole blood Se content (BSe) and the activity of the seleno-enzyme, glutathione peroxidase (GSHpx) (*EC* 1.11.1.9). Healthy neonates, children up to 15 years and children with malabsorption states, treated epilepsy and asthma were studied.

Cord blood BSe concentrations were high in neonates but fell during infancy and rose to constant levels by age 3 years. GSHpx increased gradually over the same period. Consequently, a reference value of (mean+SD) 118·5±17·4 μg BSe/l was calculated for healthy children (*n* 50) aged 3 to 14 years. GSHpx in these children was (mean±SD) 21·7±2·6 units/g Hb.

The BSe level in children with coeliac disease was significantly lower (92·4±17·4 μg/l, *n* 11, $P<0.001$) than the reference values but GSHpx activity (24·9+3·4 units/g Hb) was not significantly different from the reference range. Similarly, children with cystic fibrosis had significantly lower BSe levels (76·6+22·9 μg/l, *n* 22, $P<0.001$) than healthy children, but GSHpx, (18·9±6·73 units/g Hb) was not significantly different from the reference group.

BSe concentrations in asthmatics and epileptics conformed to the reference range, but GSHpx in these groups was 34·5±5·6 and 32·53±8·2 units/g Hb respectively, both of which were significantly higher ($P<0.001$) than in healthy children.

The increase in GSHpx activity during early infancy may reflect adaptation to extra-uterine oxidative stress (Rudolph & Wong, 1978). Our results indicate a reduced Se status in coeliac disease and cystic fibrosis, but the preservation of GSHpx suggests more efficient use of body Se, and is consistent with the findings of Lloyd-Still & Ganther, 1980.

The interesting finding of raised GSHpx in the children with epilepsy and asthma suggests induction of the enzyme, possibly by oxidative stress, but this remains to be elucidated. We feel that the above factors should be considered when using GSHpx to assess human Se status.

The clinical importance of these results requires cautious evaluation, since no child had features of Se deficiency, and the BSe and GSHpx values obtained in both the healthy and the disease states exceeded those of healthy children from New Zealand (McKenzie *et al.* 1978).

Lloyd-Still, J. D. & Ganther, H. E. (1980). *Pediatrics* **65**, 1010.
McKenzie, R. L., Rea, H. M., Thomson, C. D. & Robinson, M. F. (1978). *Am. J. clin. Nutr.* **31**, 1413.
Rudolph, N. & Wong, S. L. (1978). *Pediat. Res.* **12**, 789.

The effect of sub-clinical iron deficiency on lead absorption. By J. N. MORRISON and J. QUARTERMAN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Lead absorption in the rat is known to be increased by iron deficiency when this is severe enough to produce marked decreases in haemoglobin concentration and haematocrit (Hamilton, 1978). Fe deficiency in human subjects may be sufficiently mild to decrease Fe stores but not to influence these haematological characteristics and it is important to know if such a degree of Fe deficiency could increase Pb absorption.

Rats weighing 300 g were given a semi-purified diet containing 1·5 μg Fe/g for 7 d. At the end of this time there was no change in body-weight, haematocrit or haemoglobin concentration when compared to control animals receiving the same diet supplemented to contain 50 μg Fe/g. Solutions (0·5 ml) containing 2·5 μCi ^{59}Fe and $1{\cdot}8\times10^{-5}$ mol Fe or 3·0 μCi ^{203}Pb and $2{\cdot}4\times10^{-5}$ mol Pb in physiological saline were injected into ligated loops of proximal intestine under phenobarbital anaesthesia and the absorption into the carcass after 2 h measured in a whole body counter after the removal of the loop.

The absorption of both Fe and Pb was increased by this short period on a low-Fe diet by 100 and 60% respectively. Intestinal loops of low-Fe animals retained 50% less ^{59}Fe and 30% more ^{203}Pb than those of control animals.

				Injected dose (%)			
				Transported to carcass		Bound to loop	
Treatment group	Body-weight	Haemoglobin (g/100 ml)	Packed cell volume (%)	Fe	Pb	Fe	Pb
Fe supplemented	292±7	17·6±0·3	54·6±0·7	28±4	4·9±0·6	54±4	40±3
Fe deficient	294±4	17·3±0·2	53·7±0·6	58±3	8·1±1·1	26±1	52±5

A model system has now been developed in which rats given a low Fe diet, slowly develop symptoms of Fe deficiency, first indicated by a decreased liver non-haem Fe and only at a late stage producing anaemia. By determining the point in this process at which Pb absorption starts to increase, the relationship between Fe deficiency and Pb absorption may be clarified.

Hamilton, D. L. (1978). *Toxic. appl. Pharmac.* **46**, 651.

Treated straws and turnips in rations for beef steers. By P. E. V. WILLIAMS and A. MACDEARMID, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Improvements in the nutritive value of cereal straws following chemical treatment (Sundstøl *et al.* 1978; Ørskov, 1979) have increased the potential use of straw in diets for ruminants. In a previous experiment to assess the nutritive value of treated straws in a diet with turnips, daily live-weight gains and feed intakes declined and straw digestibility and rumen pH were low in steers offered the mixed turnip and straw diet. This may have been due to the soluble carbohydrate present in the turnips depressing rumen cellulolytic bacterial activity. To investigate the effect of turnips on the intake and digestion of straws by cattle the degradability of caustic treated (CS), anhydrous ammonia treated (AS) or untreated barley straw (US) was examined in two cannulated steers fed basal diets of either caustic treated straw plus urea, caustic straw and urea plus an allowance of chopped turnips (50 g turnip DM/kg $W^{0\cdot75}$) or turnips alone. The steers were given the basal diets for six week periods, over the last two weeks of each period the degradability of the straws was determined by the dacron bag technique (Ørskov *et al.* 1980).

Period of incubation (h)	Basal diet: Straw CS	Straw AS	Straw US	Straw + turnips CS	Straw + turnips AS	Straw + turnips US	Turnips CS	Turnips AS	Turnips US	Significant differences: Basal diet	Straw treatment
17	58	39	23	52	22	17	48	21	12	NS	***
72	77	53	29	68	45	26	62	33	19	***	***

The mean percentage dry matter degradability of the treated straw incubated for 17 or 72 h in the rumen of steers given the basal rations is shown in the Table. Treated straws (CS and AS) were degraded more at both 17 and 72 h compared with untreated straw. The degradability of all three straws was lowest with turnips as the basal diet. Turnips offered with straw depressed the degradability at 72 h of all three straws compared with the value obtained when the basal diet was straw alone. Rumen pH fell from 7·5 to 6·1 4 h after the turnip allowance was offered when the steers were on the mixed ration. Substituting an equivalent amount of soluble carbohydrate in the form of molasses for the turnips had a similar effect on straw degradability and rumen pH.

Ørskov, E. R. (1979). *Rowett Res. Inst. Rep.* **35**, 109.
Ørskov, E. R., Hovell, DeB. F. D. & Mould, F. (1980). *Trop. Anim. Prod.* **5**, 195.
Sundstøl, F., Coxworth, E. & Mowat, D. N. (1978). *Wld Anim. Prod.* **26**, 13.

Energy retention and utilization of grass and legume silage by cattle. By H. F. TYRRELL[1], D. J. THOMSON[2], D. R. WALDO[1] and H. K. GOERING[1], [1]*Ruminant Nutrition Laboratory, Animal Science Institute, Beltsville Agricultural Research Centre, Beltsville, Maryland* 20705, *USA* and [2]*Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR*

Grass (G) (cocksfoot, *Dactylis glomerata* L.) and legume (L) (lucerne, *Medicago sativa* L.) forages in the primary phase of growth, were conserved as direct-cut, unwilted silage in concrete stave towers, using both formic acid (3·5 l/tonne fresh weight) and formaldehyde (3 per 100 g crude protein) in the preservation of the crops. Each forage was harvested at two stages of maturity, early (E) and 14 d later (L), and they were designated, grass (G_E and G_L) and legume (L_E and L_L).

Within each maturity the grass and legume silages were of equivalent apparent energy digestibility (0·644 early; 0·559 late) and were offered to Holstein steers (average body-weight 265 kg) at one level of feeding (70 g DM/kg body-weight$^{0.75}$ per d) in a replicated 4×4 Latin Square experiment in which faecal, urine, methane losses, heat production and energy retention were measured in open-circuit respiration calorimeters. The cell wall content (CWC) and nitrogen (N) contents were 396 and 39; 515 and 32; 654 and 32 and 722 and 24 (g/kg DM) for silages L_E, L_L and G_E and G_L respectively. The digested cell walls contributed 0·48 to the digested organic matter in the L forage, compared with 0·88 in the grass forage. The results for k_f (efficiency of utilization of ME for growth and fattening) in the Table demonstrate a marked superiority in favour of the legume compared with the grass silage.

	Silage				
	L_E	L_L	G_E	G_L	SE
Metabolizable energy	0·534	0·463	0·538	0·465	0·004
Metabolizable energy content (MJ/kg DM)	11·0	9·2	11·2	9·7	—
Gross energy intake (MJ/d)	92·8	91·7	97·2	92·8	1·37
Metabolizable energy intake (MJ/d)	49·6	42·5	52·3	43·2	0·99
Energy retention (from carcase anaylsis; MJ/d)	6·19	2·22	5·94	2·01	0·945
k_f*	0·554	0·535	0·428	0·421	0·022

*k_f, the efficiency of utilization of metabolizable energy for growth and fattening, calculated using a within experiment maintenance requirement of 586 kJ/kg body-weight$^{0.75}$ per d.

https://doi.org/10.1079/PNS19820014 Published online by Cambridge University Press

The voluntary intake, growth rate and tissue retention of cattle fed grass or legume silage. By D. R. WALDO[1], D. J. THOMSON[2], H. K. GOERING[1] and H. F. TYRRELL[1], [1]*Ruminant Nutrition Laboratory, Animal Science Institute, Beltsville Agricultural Research Centre, Beltsville, Maryland* 20705, *USA* and [2]*Grassland Research Institute, Hurley, Maidenhead, Berkshire SL6 5LR*

Four silages described in another communication (Tyrrell *et al.* 1982), grass early (G_E) and late (G_L) and legume early (L_E) and late (L_L) maturity, prepared from cocksfoot (*Dactylis glomerata* L.) and lucerne (*Medicago sativa* L.) and harvested from the primary phase of growth, were each fed *ad lib.* to eight Holstein steers (initial body-weight 220 kg) for 130 d. Forage, preserved as silage with formic acid and formaldehyde, was the sole food and was offered once daily. The initial composition and energy content of the cattle was estimated from a sample slaughter group (8), and the final values were measured by chemical analysis of the minced carcass (C) and non-carcass (NC) tissues.

When offered the legume compared with the grass silage cattle voluntarily consumed more digestible energy ($P<0.01$), grew faster ($P<0.01$), (those in the L_E silage in excess of 1 kg live-weight gain/d) and retained more protein, fat and energy, in the carcass ($P<0.01$) and the total body ($P<0.01$).

	Silage				
	L_E	L_L	G_E	G_L	SE
Metabolizable energy intake (MJ/d)	81·7	64·3	63·8	47·9	2·15
Live-weight gain (g/d)	1041	903	859	613	29·1
Empty body-weight gain (g/d)	992	784	692	430	28·6
Carcass protein retention (g/d)	113	92	91	55	4·4
Carcass energy retention (MJ/d)	9·19	5·68	5·55	2·98	0·321
Total body protein energy retention (MJ/100 MJ total body energy retention)	38·1	51·2	50·3	75·3	8·56

Gut fill (% live weight) was 15·6, 20·1, 21·6 and 24·8 for diets L_E, L_L, G_E and G_L respectively, and the values protein energy:total body energy retained were 0·381, 0·512, 0·503 and 0·753 for the four diets respectively. The higher retention of protein, fat and energy by cattle fed on legume compared with grass silage could be attributed to both a higher voluntary intake of food and an enhanced efficiency of energy utilization (Tyrrell *et al.* 1982). The legume (lucerne) would yield, per hectare, an amount of DM similar to the grass (cocksfoot) receiving 300 kg N fertilizer/ha, but 61% additional edible (C) protein and 30% additional edible (C) energy/ha would be available for human consumption from the legume compared with the grass.

Tyrrell, H. F., Thomson, D. J., Waldo, D. R. & Goering, H. K. (1982). *Proc. Nutr. Soc.* 41, 23A.

The relationship between energy metabolism and blood level of thyroid hormones during early lactation of dairy cows. By G. BERTONI, G. PALLAVICINI and ROSANNA LOMBARDELLI, *Istituto de Zootenica, Facolta di Agraria, Universita Cattolica S. Cuore,* 29 100 *Piacenza, Italy*

Although the importance of thyroid hormones during lactation has been established, a negative relationship between milk production and the level of these hormones in blood has been described (Cappa & Bertoni, 1971; Hart *et al.* 1978) In order to determine the cause of this relationship two sets of experiments were carried out. In the first thirteen dairy cows in the last 10–15 d of pregnancy and in the first 35 d of lactation were used to measure milk output and the plasma concentrations of glucose, ketone bodies, T_3, T_4, and RT_3. In the second experiment ten cows (four with low and six with normal energy intake) were subjected to a dynamic test of the thyroid regulating hormone (TRH; intravenous injection of 500 μg with frequent blood sampling up to 48 h). Both groups contained animals in early (15–30 d) and late (120–150 d) lactation.

In early lactation there was a negative relationship between T_3 and milk production (r −0·33, $P<0·01$), but only in low producing cows. In high producing cows there was a positive correlation between levels of T_3 and plasma concentrations of glucose (r 0·30, $P<0·05$). The levels of T_3 and T_4 always increased in response to TRH injection. However, the response was slightly more marked in early lactation (especially in animals with low energy intake).

The results indicate that thyroid activity is depressed during lactation even when energy intake is low. This may be a mechanism for conservation of glucose for use by the mammary gland.

Cappa, V. & Bertoni, G. (1971). *Folia Vet. latina* **1**, 552.
Hart, I. C., Bines, J. A., Morant, S. V. & Ridley, J. L. (1978). *J. Endocr.* **77**, 333.

Effects of sodium bicarbonate or sodium chloride or both upon performance of weaned calves. By G. C. OKEKE and J. G. BUCHANAN-SMITH, *Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1*

The addition of sodium bicarbonate to the diets of growing cattle and lactating cows increased the rumen turnover rate of soya-bean meal and enhanced the disappearance of soya-bean meal protein from nylon bags suspended in the rumen. The degradability of soya-bean meal protein in the rumen, estimated by the method of Ørskov & McDonald (1979), was therefore unaffected (Okeke & Buchanan-Smith, 1981). It was found that the rumen pH (elevated by sodium bicarbonate) was positively associated with degradability. Therefore, to determine whether the protein nutrition of ruminants could be influenced by the acid:base reaction of the salt, sodium bicarbonate was compared with sodium chloride as a supplement in the diet of growing calves. Forty-eight Holstein-Friesian calves, mean weight 83 kg, were fed on a diet based upon (g/kg DM) cracked maize 720, maize silage 100, and chopped oat straw 60. Calves were assigned randomly to six treatments: control, no supplement and salts (see Table). Calves were fed individually *ad lib.* for three 4-week periods. In the first two periods, the crude protein content of the diet was 14% and in the last period it was 11%, on a DM basis. Salts replaced cracked maize. Protein in the diets was fortified with urea (5 g/kg DM in all diets) and soya-bean meal.

	Control	$NaHCO_3$ (g/kg DM)		NaCl (g/kg DM)		$NaHCO_3$ (15 g/kg DM) and NaCl (10·5 g/kg DM)	SEM
		15	30	10·5	21		
12 weeks							
Daily gain (kg/d)	1·02	1·15	1·03	1·12	1·11	1·11	0·032
DM intake (kg/d)	3·69	4·03	4·06	3·87	4·01	4·04	0·149
DM intake/daily gain	3·61	3·52	3·93	3·46	3·62	3·64	0·092
5th–8th week							
Daily gain (kg/d)	0·91	1·22*	1·12	1·22*	1·19*	1·25*	0·030
DM intake (kg/d)	3·82	4·21	4·45	4·13	4·35	4·31	0·110
DM intake/daily gain	4·22	3·45*	3·98	3·39*	3·66*	3·46*	0·085

*Superior to control, $P<0·05$.

The results suggest that both salts can improve growth rate and feed efficiency in calves. It is possible that this result could be due to another effect upon protein digestion such as an improvement in microbiol efficiency (Harrison & McAllan, 1980).

Harrison, D. G. & McAllan, A. B. (1980). In *Digestive Physiology and Metabolism in Ruminants*, p. 205 [Y. Ruckebusch and P. Thivend, editors]. Lancaster: MTP Press Ltd.

Okeke, G. C. & Buchanan-Smith, J. G. (1981). *Fedn Proc. Fedn Am. Socs exp. Biol.* **40**, 875.

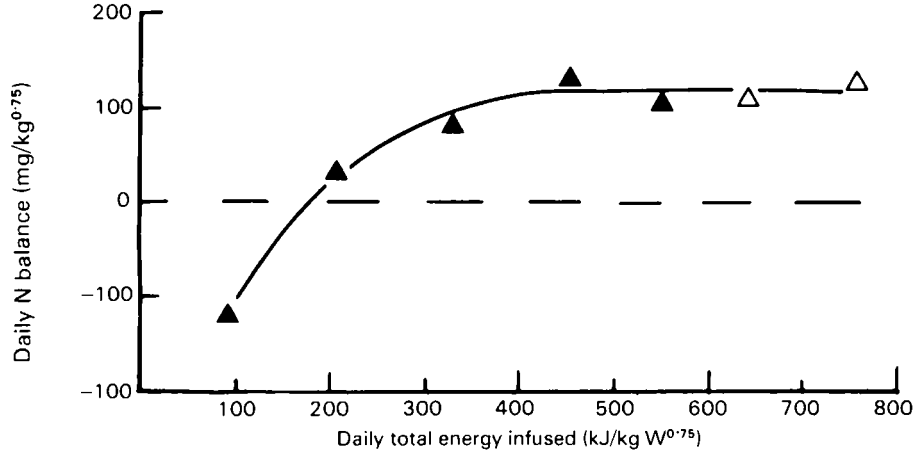
Ørskov, E. R. & McDonald, I. (1979). *J. agric. Sci. Camb.* **92**, 499.

The effect of energy intake on nitrogen retention by lambs totally nourished by intragastric infusion. By F. D. DEB. HOVELL, N. A. MACLEOD, R. A. STIRTON and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The development of the intragastric infusion technique of alimentation has enabled the nitrogen and energy metabolism of ruminants to be studied independently of the effects of the activity of the rumen microbial population. Recent work (Ørskov & Grubb, 1978) has shown that the requirement for the maintenance of N equilibrium in ruminants is considerably higher than that suggested by the Agricultural Research Council (ARC) (1980). Ørskov & Grubb (1978) found the endogenous urinary N (EUN) of lambs given 450 kJ/kg $W^{0.75}$ per d as volatile fatty acid (VFA) directly into the rumen as their sole energy source to be 429±32 mg N/kg $W^{0.75}$ per d (mean of five animals measured over 9 d). The object of this experiment was to study the effect of energy, supplied as VFA infused into the rumen, on the N retention by lambs when given protein (casein) as an abomasal infusion in amounts estimated to be approximately 15% in excess of that needed to satisfy the maintenance requirement for N as defined by EUN excretion (0·8 utilization of casein assumed).

Four lambs weighing between 30–35 kg fitted with rumen and abomasal cannulas were given 641 mg N/kg $W^{0.75}$ per d as casein infused directly into the abomasum. A VFA mixture containing 0·65, 0·25, 0·10 molar proportions of acetic, propionic and butyric acids respectively, was infused into the rumen, the rates ranging from about 115 to about 700 kJ/kg $W^{0.75}$ per d; in one further period no VFA were infused (first point on the Figure).

The Figure shows the main results. Each point represents the mean of three observations of three (△) or four (▲) lambs for the last 3 of 5 d at each energy level. N balance became positive at about one-half energy maintenance. This result has clear implications for the practical feeding of ruminants. Creatinine excretion showed no clear trend with time, average excretion being 60 mg/kg $W^{0.75}$ per d equivalent to about 24·9 mg/kg live weight.



Agricultural Research Council (1980). *The Nutrient Requirements of Ruminant Livestock.* Farnham Royal: Commonwealth Agricultural Bureaux.

Ørskov, E. R. & Grubb, D. A. (1978). *Proc. Nutr. Soc.* **38**, 24A.

The effect of an anabolic implant (trenbolone acetate + oestradiol-17β) on the metabolic rate and protein metabolism of beef steers. By G. E. LOBLEY, J. S. SMITH, G. MOLLISON, ALEXMARY CONNELL and H. GALBRAITH, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB* and *North of Scotland College of Agriculture, Aberdeen*

Anabolic implants, especially those based on the synthetic steroid trenbolone acetate given alone or in combination with an oestragen, are widely used in the UK to finish beef steers, but as yet the mode of action for these steroids is ill-defined. This preliminary report describes the effect of one preparation (140 mg trenbolone acetate+20 mg oestradiol-17β; 'Revalor', Hoechst, UK) on metabolic rate and protein metabolism in beef steers.

Six Hereford × Friesian steers (1 year old, 230–250 kg) were accustomed for 3 months to a diet (ruminant diet AA6) delivered from continuous belt feeders. The ration was adjusted on a live weight basis every 3 weeks to provide a calculated ME intake of 1·6×maintenance. The experiment started when the steers reached 300 kg live weight. On six occasions, at 3 week intervals, each steer underwent a 6 d energy and nitrogen balance inside automated confinement respiration chambers. One week after the second 6 d balance, three of the steers were implanted with Revalor, while the three control animals were sham-implanted.

The Revalor-implanted steers showed a significant improvement ($P<0·001$) for both live-weight gain (1·11 *v.* 0·69 kg/d) and feed conversion efficiency (0·13 *v.* 0·08) compared with the controls during the 11 weeks post-implantation, but this was not associated with any change in digestibility or metabolizability of the diet. Heat production was also similar for both groups when measured at the same weights and intakes of ME and in consequence, there was no difference between groups in the total amount of energy retained, although individual animals did exhibit some variation in their efficiency of utilization of ME. As N retention during the experimental period was greater in the treated steers (43·7 *v.* 24·7 g N/d, $P<0·001$) the fraction of total energy apparently retained as protein was higher than in the controls (0·40 *v.* 0·28, $P<0·05$).

Protein synthesis and amino acid oxidation were measured on day 3 or 4 of each chamber period when the steers were continuously infused for 8–9 h with [1-^{14}C]leucine (10 μCi/h). There were differences between individual steers in their rates of protein synthesis (26–36 g protein synthesized/kg $W^{0·75}$ per d) and these persisted throughout the study. There was no detectable effect of the steroids on the rate of protein synthesis but 2 weeks after implantation the two steers which exhibited the greatest anabolic response had decreases in their fractional rate of oxidation (0·08–0·04). It was calculated that this represented a reduction in protein oxidation of 90–110 g. It was not until 6 weeks later that leucine catabolism had returned to preimplantation values.

Effect of the feeding level of grass on the rate of outflow of protein supplements from the rumen of sheep. By M. E. ELIMAM and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The rate of outflow of protein supplements from the rumen is an important factor affecting the extent to which the supplements are degraded by the rumen micro-organisms (Ørskov & McDonald, 1978). The extent of degradation of protein in the rumen must be known in order to estimate the contribution of undegraded dietary protein to the protein economy of the host animal. The outflow rate of the protein of a supplement has been measured by marking a 'dose' of the protein with Cr, and measuring the rate at which Cr appeared in the faeces (Elimam & Ørskov, 1980). The work reported here was undertaken to investigate the effect of the feeding level of a forage (grass) on the rate of outflow from the rumen of supplemental soya-bean meal or white fish meal.

Four sheep were given chopped dried grass at four levels of intake (0·5, 1·0, 1·5 and 2·0 times energy maintenance). A 4×4 Latin Square design was used, and each of the two treated protein supplements was given as a single oral dose once within each period. The fractional outflow rate of the supplement from the rumen increased in proportion with the increase in feed intake (see Table). There were no significant differences between supplements, but there were differences between animals. The fractional outflow rate from the rumen of one sheep was about 40% greater than that in the other three animals.

The effect of feeding level on the fractional outflow rate/h of Cr-treated protein supplements determined indirectly from the concentration of faecal Cr

Feeding level (multiple of maintenance)	White fish meal	Soya-bean meal	Combined
0·5	0·010	0·010	0·010
1·0	0·019	0·026	0·022
1·5	0·032	0·034	0·033
2·0	0·038	0·039	0·039
SE of differences	0·0032	0·0032	0·0023

The outflow rate of protein determined the proportions of protein degraded. For example, Ørskov *et al.* (1980) calculated that the proportion of soya-bean meal degraded within the rumen would be reduced from 0·89 at a fractional outflow rate of 0·01/h to 0·68 at a fractional outflow rate of 0·04/h.

Elimam, M. E. & Ørskov, E. R. (1981). *Anim. Prod.* (In the Press).
Ørskov, E. R. & McDonald, I. (1978). *J. agric. Sci., Camb.* **92**, 499.
Ørskov, E. R., Hughes-Jones, M. & McDonald, I. (1980). *Recent advances in animal nutrition*, p. 85 [W. Haresign and D. Lewis, editors]. Butterworths.

Maternal energy intake in pregnancy and its relation to maternal foetal factors. By DORIS M. CAMPBELL, B. M. CAMPBELL-BROWN, L. JANDIAL and I. MACGILLIVRAY, *Department of Obstetrics and Gynaecology, University of Aberdeen, Forester Hill, Aberdeen AB9 2ZB*

There is continuing interest in the association of maternal nutrition with birth weight because of the known association between low and high birth weights and perinatal death. Studies were made of the relationships between maternal nutritional intake and the outcome of pregnancy in two different groups of Aberdeen primigravidae, one at risk of having a light-for-dates infant (Group 1) and the second an obese population (Group 2). The mean energy intake for these two groups was almost identical (Campbell *et al.* 1979).

Group 1 was selected when any two of the following four characteristics were present: height less than 1·54 m, weight at 20 weeks less than 54 kg, weight for height less than 25th centile (Kemsley *et al.* 1962), weight gain between 20 and 30 weeks less than 3·3 kg, and Group 2 were primigravidae whose weight for height was greater than the 75th centile.

In this study the mean energy intake was 8·78 MJ (2090 kcal)/d in Group 1 and 8·45 MJ (2013 kcal)/d in Group 2 in spite of considerable differences in body size and birth weight. Maternal height, maternal weight at 20 weeks, birth weight and birth weight centile have been compared in these two groups according to their daily energy intake.

	Mean daily energy intake (MJ/d)			
	<6·72	6·72–8·39	8·40–10·07	≥10·08
Distribution (% (*n*))				
Group 1 (88)	6·8 (6)	34·0 (30)	40·9 (36)	18·2 (16)
Group 2 (63)	15·8 (10)	38·1 (24)	27·0 (17)	19·1 (12)
Mean daily energy intake (MJ)				
Group 1	5·99	7·59	9·13	11·29
Group 2	5·28	7·48	9·33	11·94
Mean height (m)				
Group 1	1·50	1·54	1·56	1·57
Group 2	1·58	1·58	1·62	1·61
Mean weight (kg) at 20 weeks				
Group 1	51·0	50·7	50·5	50·3
Group 2	76·1	71·1	72·0	76·3
Mean birth weight (g)				
Group 1	2845	2985	3059	2948
Group 2	3235	3241	3350	3286
Birth weight <10th centile				
Group 1	33·0	27·0	19·0	25·0
Group 2	20·0	12·5	0	16·7
Birth weight <25th centile				
Group 1	66·0	57·0	44·0	69·0
Group 2	20·0	25·0	11·8	25·0

Campbell, D. M., Campbell-Brown, B. M., Jandial, L. & MacGillivray, I. (1979). *Proc. Nutr. Soc.* **38**, 53A.

Kemsley, W. F. B., Billewicz, W. Z. & Thomson, A. M. (1962). *Br. J. Prev. Soc. Med.* **16**, 189.

Energy cost of deposition and maintenance of 'obese tissue' in the adult rat. By K. J. McCracken, *Department of Agriculture, N. Ireland* and *The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX* and Mary A. McNiven, *Swedish University of Agricultural Sciences, Uppsala, Sweden*

There are conflicting reports as to the relative contributions of lean body mass and 'obese tissue' to the energy requirements of normal and obese subjects (Bray, 1976; Garrow, 1978). It is probable that the differences found are due on the one hand to the types of obese subject studied and to the methods used to estimate lean body mass. The force-fed adult rat provides a model for investigating the relationship between body composition and metabolic rate which is not subject to these difficulties. In a previous study (McCracken & Gray, 1976) adult female rats were fed for 64 d, during which time weight increased from 220 to 370 g, the fasting heat production being measured at intervals. This study suffered from the possible carry-over effects of previous fasting periods. The experiment described below was designed to avoid this problem.

Thirty-six ten-month-old Norway Hooded rats, blocked for weight, were over-fed a synthetic diet (g/kg: casein 120, fatted skim milk 150, starch 310, sucrose 250, vegetable oil 120, minerals/vitamins 50) by gastric intubation. Room temperature was 30°. On specified days fasting heat production (FHP) was measured on six rats for 24 h before slaughter for carcass analysis.

	0	6	12	18	24	30	Statistical significance	SE of a difference
Weight (g)	244	263	285	311	338	365	•••	4·4
Crude protein (g)	44·6	45·6	45·6	48·5	48·1	48·5	••	1·15
Fat (g)	53·7	60·2	85·7	106·7	136·2	163·0	•••	6·15
FHP (kJ/d)	118	125	132	139	147	160	•••	4·9
FHP (kJ/kg $W^{0·75}$ per d)	336	337	333	330	329	338	NS	12·2

During the 30 d period the weight of the rats increased by 50%, protein by 9% and fat by 200% (Table 1). Fasting heat production and metabolic body size (kg $W^{0·75}$) increased by 36%. The mean metabolizable energy intakes (MJ/rat) of groups day 6–day 30 respectively were 1·46, 3·24, 5·29, 7·28, 9·18. Assuming k_m=0·90 (McCracken & Gray, 1976) the mean efficiency of energy utilization for fattening (k_f) was calculated to be 0·84.

It is concluded that (1) adult rats made obese by gastric intubation do not exhibit 'diet-induced thermogenesis' other than that due to increased body size; (2) increased fasting heat production is closely correlated to metabolic body size but not to lean body mass.

Bray, G. (1976). *The Obese Patient.* Philadelphia: Saunders.
Garrow, J. S. (1978). *Energy Balance and Obesity in Man.* Amsterdam: Elsevier/North-Holland.
McCracken, K. J. & Gray, R. (1976). *EAAP Publ. No.* **19**, 141.

Thermic response to fat feeding in lean and obese subjects. By C. A. ZED and W. P. T. JAMES, *MRC Dunn Clinical Nutrition Centre, Old Addenbrooke's Hospital, Cambridge CB2 1QE*

Studies in both obese and normal weight individuals have demonstrated a reduction in energy expenditure on semi-starvation diets (Keys *et al.* 1950; Shetty *et al.* 1979). Overfeeding studies in lean subjects (Goldman *et al.* 1975) have also shown adaptation in metabolic efficiency with a marked increase in heat production. A change in metabolic efficiency may involve changes in the thermic response to food. It has been shown previously that the postprandial thermic response to a mixed meal is subnormal in obesity (Shetty *et al.* 1981), but the response to a protein or a carbohydrate meal is similar in lean and obese subjects and unaltered by energy restriction (Zed & James, 1980). In this study the thermic response was measured over 24 h to determine whether lean and obese subjects respond similarly to a fat supplement while on a weight maintenance diet, and whether this response was altered when the same supplement was reintroduced after a period of energy restriction.

Eight obese and eight lean subjects were studied, with mean heights of $1{\cdot}618\pm0{\cdot}041$ m and $1{\cdot}643\pm0{\cdot}094$ m respectively, mean weights of $116{\cdot}5\pm21{\cdot}1$ kg and $50{\cdot}2\pm5{\cdot}7$ kg and mean ages of $37{\cdot}4\pm8{\cdot}1$ yrs and $25{\cdot}8\pm7{\cdot}6$ yrs. The same diet, which contained 10 MJ/d (protein–fat–carbohydrate, 16:42:42) was fed to all subjects for 6 d, after which a fat supplement of 4·2 MJ/d was given for a further 6 d. In each phase, two 24 h measurements of heat production were made in a whole body calorimeter and the increased expenditure calculated in terms of the energy of the additional fat fed. In six of the obese and five of the lean subjects there followed a 10 d period in which energy was restricted to 5 MJ/d (protein–fat–energy, 33:33:33), with equivalent reductions in the intake of fat and carbohydrate; the 4·2 MJ/d fat supplement was reintroduced for the final 6 d of the study.

The fat supplement following the 10 MJ/d diet caused a greater thermic response in lean (542 kJ) than in obese (213 kJ) subjects ($P<0{\cdot}0025$). These increases represent 13·1 and 5·1% respectively of the energy content of the fat supplement. However, when the supplement was reintroduced after 10 d of energy restriction the thermic response was reduced to 255 kJ in lean and 193 kJ in obese subjects and there was no longer any significant difference between the groups.

These results suggest that thermic adaptation to meal feeding relates to its fat content and that in obesity there is a subnormal response.

Goldman, R. F., Haisman, M. F., Bynum, G., Danforth, E. Jr, Horton, E. S. & Sims, E. A. H. (1975). In *Obesity in Perspective,* Md. 2 (pt. 2), 165. Bethesda: National Institute of Health.

Keys, A., Brozeck, J., Henschel, A., Mickelson, O. & Taylor, H. L. (1950). *The Biology of Human Starvation,* vol. 1. Minneapolis: Univ. Minnesota Press.

Shetty, P. S., Jung, R. T. & James, W. P. T. (1979). *Lancet* i, 77.

Shetty, P. S., Jung, R. T., James, W. P. T., Barrand, M. A. & Callingham, B. A. (1981). *Clin. Sci.* **60,** 519.

Zed, C. & James, W. P. T. (1980). *Alim. Nutr. Metab.* **1,** 385.

Effect of litter size and post-weaning high-sucrose diet on development of obesity in the rat. By E. KRIZ (introduced by K. ANANTHARAMAN), *Research Department, Nestlé Products Technical Assistance Co. Ltd, CH-1814 La Tour-de-Peilz, Switzerland*

The role of early nutrition remains an open question especially in relation to the etiology of obesity (Knittle & Hirsch, 1968; Harris & Widdowson, 1978; Cryer & Jones, 1979).

We have studied the influence of high and low level of early nutrition on the development of obesity due to a high-sucrose diet in Sprague-Dawley rats (Charles-River, France).

Male rats were raised in litters of four (high level of nutrition, HL), ten (normal level, NL) or sixteen (low level, LL) and fed *ad lib.* a control (C; no sucrose) or high-sucrose (HS; 500 g sucrose/kg), balanced semi-synthetic diet from weaning. Randomly chosen animals were killed at 6, 9 and 13 weeks of age and adipose tissue (subcutaneous inguinal, epididymal and perirenal) was collected. Plasma insulin and glucose and adipocity (Coulter Counter) were determined. Records of individual weekly body-weight and food intake were maintained.

Early HL increased body fat (obesity), compared to NL animals. The effect was significant on diet C though on diet HS it was partially masked by the development of obesity due to the diet itself. Obesity induced by early HL developed without postweaning hyperphagia on diet C whereas, early LL diminished the development of obesity on diet HS and did not affect animals on C.

Body-weight differences between groups were due to the development of adipose tissue alone, lean body mass remaining nearly unchanged. Early nutrition states resulted in differential effects in the three adipose tissue sites. Thus, early HL especially stimulated the inguinal tissue, whilst early LL more or less equally decreased development of all three sites with a greater decrement of the perirenal tissue.

Development of adipose tissue was mainly hypertrophic (increased cell volume), the small increase in cell number in inguinal adipose tissue of early HL animals being not significant.

The level of immunoreactive insulin (13 weeks) correlated positively with the group differences in body fat, resulting from pre- and postweaning nutritional situations. Blood glucose level remained unchanged.

The results confirm that in rats early HL is implicated in the induction of obesity. Even on diet C, HL animals became obese without showing postweaning hyperphagia. Apparently this predisposition to obesity is in relation to or a consequence of a precocious accelerated development due to preweaning HL. However, early LL (moderate) seems to protect against the development of obesity due to diet HS, at least up to 13 weeks.

Cryer, A. & Jones, H. M. (1979). *Biochem. J.* **178**, 711.
Harris, P. M. & Widdowson, E. M. (1978). *Br. J. Nutr.* **39**, 201.
Knittle, J. L. & Hirsch, J. (1968). *J. clin. Invest.* **47**, 2091.

Energy balances of humans at three levels of metabolizable energy intake. By A. J. H. VAN ES and J. E. VOGT, *Department of Animal Physiology,* P. DEURENBERG, *Department of Human Nutrition, Agricultural University, Wageningen* 6709 *PJ* and E. J. VAN DER BEEK, *Central Institute for Nutrition Research, Zeist,* 3700 *AJ*

Few 24 h energy balances of humans are available. Therefore, in a cooperative effort a series of energy balance experiments were performed using two large-animal respiration chambers from the Department of Animal Physiology. The chambers were converted into small (11 m^3) hotel rooms, equipped with bed, washstand, desk, chairs, TV, radio, telephone, intercom, exercise bicycle and weighing scales. The chambers had two small windows in the outer walls, and a $0{\cdot}8 \times 0{\cdot}8$ m window in the common inner wall.

Energy and N balances of seven volunteers, four males of 19–55 years and three females of 19–22 years were measured at ME intakes of about 4·5, 9·0 and 13·5 MJ. One trial lasted 7 d. The diet used consisted of sixteen ingredients, each was weighed and analysed for energy and N. Faeces and urine were collected and analysed for the last 4 d. The volunteers entered the chambers on the fourth day, gas exchange was measured from the next morning onwards for 3×24 h. Intervals of 1–3 weeks separated the trials with each volunteer. Volunteers were asked to follow a standard activity programme including breakfast, lunch and dinner, five periods of 15 min on the exercise bicycle, office work or leisure and bedrest from 23.00 till 08.30 hours. The chambers were kept at 22° during the day and at 19° from 22.30 till 08.30 hours; relative humidity was about 0·65. Neither the diets nor the stay in the chambers were reported to give much discomfort.

Energy and N digestibilities were with two exceptions between 0·93 and 0·95, urinary losses 0·3–0·5 MJ/d. Methane production was almost absent. For the same person, heat production calculated from gas exchange was nearly equal at the lower ME intakes and only slightly higher at the highest. Intakes of metabolizable energy at energy equilibrium, obtained by interpolation, averaged 440 kJ/kg $W^{0{\cdot}75}$ with a range from 400 to 490 kJ/kg $W^{0{\cdot}75}$.

A second series with eight volunteers is reaching completion.

The support of the Netherland Heart Foundation is gratefully acknowledged as well as the cooperation of the seven volunteers, four of whom took an active part in the experimental work.

Dietary thermogenesis and exercise. By HELEN DALLOSSO and W. P. T. JAMES, *MRC Dunn Clinical Nutrition Centre, Old Addenbrooke's Hospital, Cambridge, CB2 1QE*

Exercise may contribute to 24 h energy expenditure in ways other than simply the energy cost of the activity itself—by increasing the thermic effect of food during exercise (Miller *et al.* 1967) and by a delayed return to pre-exercise energy expenditure values after heavy exercise (Edwards *et al.* 1935). Such exercise effects may be absent in the obese condition (Zahorska-Markiewicz, 1980) and enhanced during overfeeding (Stock, 1980).

Eight non-smoking weight-stable men (23±2 years, 1·78±0·06 m, 69·7±4·8 kg) lived for 14 d in the metabolic suite at the Dunn Clinical Nutrition Centre in Cambridge. For 7 d they ate a weight maintenance diet (12% protein, 30% fat) with an energy intake pre-determined individually from a 7 d weighed food intake record. They consumed 50% more energy as fat for a further 7 d, bringing the composition of their diet to 9% protein 50% fat. From 20.00 hours on the fourth and sixth days of both dietary conditions they spent 36 h in a whole body indirect calorimeter and followed a regimen of physical activity designated either High (cycling on a bicycle ergometer during six 30 min periods) or Low (only two 30 min periods). The order of presentation of the two exercise conditions was reversed for the alternate subjects. The protocol received ethical approval from the Unit's Ethical Committee.

The thermic effect of a meal (in the resting and exercise conditions) was defined as the difference (in kJ/min) between the energy expended during 30 min 1 h before and 1 h after the meal was eaten. Preliminary analysis of the data on eight subjects shows that the thermic effect of the meal was 1·51±0·42 kJ/min at rest and 1·22±0·92 kJ/min during exercise. There was no significant difference between the two conditions. In the over-feeding condition (where the meal was 50% larger) the corresponding thermic effects were 2·2±0·48 kJ/min and 2·04±0·74 kJ/min (also no significant difference). A 30 min period of cycling at 2 kp immediately before the subjects went to bed was followed by a rapid fall to base-line values with no significant effect on the total 8 h night time energy expenditure.

The interaction of exercise and food therefore is either not as important energetically as previously considered or is dependent on nutrients not used in this study.

Edwards, H. T., Thorndike, A. & Dill, D. B. (1935). *New Engl. J. Med.* **213**, 532.
Miller, D. S., Mumford, P. & Stock, M. J. (1967). *Am. J. clin. Nutr.* **20**, 1212.
Stock, M. J. (1980). *Eur. J. Appl. Physiol.* **43**, 35.
Zahorska-Markiewicz, B. (1980). *Eur. J. Appl. Physiol.* **44**, 231.

24 h oxygen consumption of rats stimulated by cafeteria feeding. By J. F. ANDREWS and B. DONNE, *Department of Physiology, Trinity College Dublin, Dublin*

Is there a specific physiological mechanism enabling dissipation of excess energy intake as heat? Studies in the rat by Rothwell & Stock (1979) have supported the existence of such diet-induced thermogenesis whilst Armitage *et al.* (1981) were unable to show its occurrence. The small excess heat production that the latter observed in cafeteria fed rats was merely the sum of the energy costs leading to fat storage. We have attempted a resolution of these conflicting observations by recording 24 h oxygen consumption as did Armitage *et al.* (1981) whilst following the cafeteria feeding protocol of Rothwell and Stock (1979).

Two groups of six animals were maintained at 30°; one group was fed on a stock diet (STOCK) and the other given a cafeteria supplement (CAF) for 4 weeks. Total 24 h oxygen consumption (Andrews & Mercer, 1973) and body-weights were measured weekly and after killing, the right interscapular brown fat pad and right epididimal fat pad weights were measured. Before commencement of cafeteria feeding total 24 h O_2 consumption (l/animal per d) was 16·0 (CAF) and 13·1 (STOCK) and the 24 h total O_2 consumption rose dramatically on cafeteria feeding to a peak of 26·6 (CAF) 16·6 (STOCK). The diet-induced thermogenesis subsided thereafter being insignificant at 28 d (CAF 20·3, STOCK 19·8 l/animal per d).

Thus our observations confirm those of Rothwell & Stock (1979); cafeteria feeding can stimulate intake which can be dissipated by diet-induced thermogenesis. The difference between observations of different groups may be the result of minor differences in protocol and of varying responses of different strains of rats.

In our experiment we cannot preclude exercise as being some component of the increased energy dissipation but it would seem improbable that it accounts for all. The half brown fat pads showed some increase in weight (212±34 mg CAF *v.* 186±23 mg STOCK; mean±SD) but due to considerable individual variation this was not significant. Thus though this experiment does not give support to the hypothesis that brown fat is the end-organ of diet-induced thermogenesis (Rothwell & Stock, 1979) it does not contradict it.

Supported by the Medical Research Council of Ireland.

Andrews, J. F. & Mercer, J. B. (1973). *J. Physiol., Lond.* **236**, 7 P.
Armitage, G., Hervey, G. R., Rolls, J., Rowe, E. A. & Tobin, G. (1981). *J. Physiol., Lond.* (In the Press).
Rothwell, N. J. & Stock, M. J. (1979). *Nature, Lond.* **231**, 31.

Metabolic responses to food, atropine and 2-deoxy-D glucose in Zucker rats. By NANCY J. ROTHWELL, M. ELIZABETH SAVILLE and M. J. STOCK, *Department of Physiology, St. George's Hospital Medical School, Tooting, London SW17 0RE*

The genetically obese Zucker rat (*fa/fa*) and mouse (*ob/ob*) both exhibit hyperphagia, but still become obese when pair-fed to their lean littermates. This suggests that low levels of energy expenditure contribute to the development of their obesity. The obese Zucker rat exhibits a reduced thermic response (TE) to food (Rothwell *et al.* 1981), but has a normal response to noradrenaline (NA). Armitage *et al.* (1980) have demonstrated that the rat's cold tolerance is also unimpaired. The present study is concerned with the influence of parasympathetic activity on thermic responses, and the effects of glucoprivation on metabolic rate.

Resting oxygen consumption ($\dot{V}O_2$) of adult lean and obese Zucker rats was measured before and after intragastric feeding of 40 kJ Complan in water. Lean animals showed a $16{\cdot}9 \pm 2{\cdot}9\%$ increase in metabolic rate in response to this meal but the rise was only $7{\cdot}2 \pm 1{\cdot}0\%$ in obese rats. These responses were reduced by β-adrenergic blockade with propanolol (10 mg/kg, subcutaneously).

Pretreatment with atropine (0·5 mg/kg, subcutaneously) caused a doubling of the TE in lean rats and a threefold increase in obese animals, such that the responses were then similar for both groups.

In anaesthetized lean rats, intragastric feeding caused a marked rise in interscapular brown adipose tissue temperature (peak increase $0{\cdot}93 \pm 0{\cdot}08°$), but in obese animals this rise in temperature was significantly ($P<0{\cdot}01$) lower ($0{\cdot}51 \pm 0{\cdot}08°$). Prior treatment with atropine caused a potentiation of the response, which was more marked in the obese ($1{\cdot}35 \pm 0{\cdot}10°$) than in the lean rats ($1{\cdot}58 \pm 0{\cdot}15°$; not significant).

Injection of noradrenaline (250 μg/kg, subcutaneously) caused similar increases in $\dot{V}O_2$ in conscious lean ($40{\cdot}9 \pm 3{\cdot}9\%$) and obese rats ($36{\cdot}2 \pm 3{\cdot}8\%$; not significant) and these were unaffected by pretreatment with atropine. However, injection of 2-deoxy-D-glucose (2 DG, 360 mg/kg, intraperitoneally) caused reductions in $\dot{V}O_2$ of $25{\cdot}5 \pm 2{\cdot}8\%$ and $7{\cdot}5 \pm 1{\cdot}3\%$ ($P<0{\cdot}001$) in lean and obese rats respectively, and pretreatment with atropine completely abolished these changes.

These results indicate that the parasympathetic nervous system may inhibit the thermic response to feeding and this inhibitory effect is much greater in the obese Zucker rat. In addition, the lower response to glucoprivation induced by 2 DG in the obese rat suggests that the obese animal is relatively insensitive to metabolic signals relating to the supply of energy.

Armitage, G., Harris, R. B. S., Hervey, G. R. & Tobin, G. (1980). *J. Physiol., Lond.* **310**, 33P.

Rothwell, N. J., Saville, M. E. & Stock, M. J. (1981). *J. Physiol., Lond.* (In the Press).

Short-term changes in urea synthesis in pigs following the addition of carbohydrate or fat to their diets. By P. J. REEDS, A. CADENHEAD, M. F. FULLER and S. M. FORBES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Eight female pigs (28–45 kg body-weight) were given a cereal based diet (Reeds *et al.* 1980) once hourly at a daily rate of 90 g/kg $W^{0.75}$. After 5 d [^{14}C]urea was infused, via a catheter inserted in the aorta, at a constant rate (3·5 μCi/h) and, after 24 h of infusion, four blood samples were removed at half-hourly intervals from a second aortic catheter. The diet was then changed to one containing additional sucrose (Diet C) or lard (Diet F) such that the intake of non-protein energy was increased by 50%. The infusion of [^{14}C]urea was continued for a further 48 h during which further blood samples were removed. Complete daily urine collections were made for 3 d before and during the period of infusion. At the end of the infusion the animals were returned to the basal diet and, after 1 week, a second experiment with the alternative supplemented diet was performed. The mean body-weights for Group C (35·1±2·3 (SE) kg) and Group F (34·8±2·6 kg) were not statistically different.

In animals offered the control diet the rate of urea entry (30·4±2·0 g urea/d) was not significantly different to the daily rate of urea excretion (28·3±2·1 g). The rate of urea entry was significantly reduced 2 d after the pigs received both supplemented diets (23·6±1·8 g/d Diet C; 22·6±2·0 g/d Diet F), but the rapidity with which the rate of urea entry changed differed between the diets.

The specific radioactivity of blood urea in pigs receiving additional carbohydrate and fat

(Values expressed relative to starting value of 100)

		Time after diet change (h)								
Group	*n*	−2	−1	0	+2	+4	+12	+24	+36	+48
C	8	99	100	100	113	117	126	133	140	142
F	7	101	99	100	102	95	90	113	151	148

Although the two supplements eventually elicited similar changes in urea synthesis, the addition of carbohydrate had a rapid and significant effect by 2–4 h, whilst the effect of the fat supplement became apparent only 24 h after it was first given. These observations suggest that carbohydrate and fat, though exerting similar protein-sparing effects, may have immediate actions upon different aspects of metabolism.

Reeds, P. J., Cadenhead, A., Fuller, M. F., Lobley, G. E. & McDonald, J. D. (1980). *Br. J. Nutr.* **43**, 445.

Protein sparing effect of caffeine during starvation. By P. J. INCH, I. A. MACDONALD, JANET L. MEAD and A. M. J. WOOLFSON, *Department of Physiology and Pharmacology, University of Nottingham Medical School, Nottingham* and *Department of Clinical Chemistry, City Hospital, Nottingham*

Nitrogen excretion during underfeeding is decreased when exogenous ketones are administered (Pawan & Semple, 1980). The present study was designed to test whether caffeine administration would potentiate the normal lipolytic response to starvation and whether any effect on protein metabolism could be produced.

Eleven normal, healthy subjects (six male, five female) took part in the experiment which was approved by the Medical School Ethical Committee. Each subject underwent two 60 h fasts; water and low energy caffeine-free drinks were taken *ad lib.* and supplements of 250 mmol NaCl and 3 mmol KCl were given. During one fast subjects took capsules containing 150 mg caffeine three times daily, in the other fast a lactose placebo was taken, the design being randomized and double-blind. All urine was collected, total volume recorded, and samples were analysed for urea and creatinine. Blood samples were taken after 12, 36 and 60 h and analysed for free fatty acid and glycerol.

		Duration of starvation (h)					
		12		36		60	
		Caffeine	Placebo	Caffeine	Placebo	Caffeine	Placebo
Plasma FFA	Mean	*633	500	1752	1577	972	1147
(μmol/l)	SD	215	181	693	440	171	350
Plasma glycerol	Mean	*93	69	235	178	117	111
(μmol/l)	SD	53	44	200	59	39	63
	n	10	10	11	11	8	8

*Significantly greater than placebo, $P<0.02$.

Eight subjects completed both periods of starvation. Fasts were terminated before 60 h in two subjects because of low blood glucose and in one subject because of nausea. Plasma FFA and glycerol concentrations were only significantly higher on caffeine fasts compared to placebo at 12 h, but, during the last 48 h of fasting in the six subjects in whom creatinine excretion was similar in both fasts, total urea excretion was significantly lower on caffeine (578 ± 93 SD mmol) than placebo (667 ± 117 mmol, $P<0.05$). This observation is supported by comparing the urea:creatinine value for the ten subjects who fasted for more than 44 h on both occasions. On caffeine fasts the ratio was significantly lower (22.4 ± 3) compared to placebo (25.8 ± 5, $P<0.05$).

It seems likely that during starvation, enhanced lipolysis with caffeine provides alternative substrates to glucose (either FFAs or ketone-bodies), thus reducing the demand for gluconeogenesis from amino acids and indirectly exerting a protein sparing effect. However, one cannot overlook the possibility that the reduced urea excretion observed in this study, resulted from some other effects of caffeine.

This work was supported in part by a grant from the Trent Regional Health Authority Clinical Research Committee to AMJW.

Pawan, G. L. S. & Semple, S. G. (1980). *Proc. Nutr. Soc.* **39**, 48A.

Effects of varying protein or food intake on catalase and superoxide dismutase activities in rat muscles. By C. J. LAMMI-KEEFE, P. V. J. HEGARTY and P. B. SWAN, *Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota* 55108, *USA*

It has been proposed that the measurement of muscle catalase activity could be used as a marker of muscle wasting (Stauber *et al.* 1977). Questions have been raised as to whether or not increased muscle catalase activity during starvation is a response primarily to a lack of energy or to the loss of muscle tissue *per se* (Lammi-Keefe *et al.* 1981). We have investigated the influence of dietary protein and the rate of muscle loss on muscle catalase and superoxide dismutase activities.

Catalase and superoxide dismutase (SOD) activities were measured in the heart and three skeletal muscles (sternomastoideus, biceps brachii and gastrocnemius) from young rats (25–41-d-old). Enzymes were measured in one group at the start of the experiment and the remainder of the animals were divided into five groups and treated as follows: fed on either 3, 6 or 25% protein diets for 16 d, totally deprived of food for 2 d, or fed on the 25% protein diet in restricted amounts for 16 d, so that their average body-weight approximated that of the rats that were totally deprived.

There were no differences in enzyme activities between the groups fed on the different protein diets for 16 d. Catalase increased in muscles when the rats lost weight due to total deprivation of food for 2 d or partial restriction for 16 d. SOD increased after total deprivation but not after partial restriction.

It is concluded that, in the young rat, muscle catalase and SOD were not altered when diets low in protein, but allowing weight maintenance, were fed over a period of 16 d. However, the increase in catalase after slow or fast weight loss confirms our previous findings for starvation in the 12-month-old rat (Lammi-Keefe *et al.* 1981) and suggests further that the rate of weight loss is not a critical factor in determining the increase. Unlike the findings for the 12-month-old rats, total starvation in the young rat also increased SOD. Thus, diets marginal in protein, but supporting weight maintenance, do not result in increased muscle catalase activity. However, food restriction, resulting in muscle weight loss, does result in increased activity. The increased enzyme activity may reflect loss of muscle *per se*, or may reflect a change in protein–energy metabolism which results in loss of muscle.

Lammi-Keefe, C. J., Hegarty, P. V. J. & Swan, P. B. (1981). *Experientia* **37**, 25.
Stauber, W. T., Bird, J. W. C. & Schottelius, B. A. (1977). *Exp. Neurol.* **55**, 381.

The effect of peripheral isotonic amino acid infusions on postoperative energy balance. By N. HARRIS, O. J. GARDEN, A. SMITH, C. C. GOLL, A. SHENKIN, R. CAMPBELL, A. J. W. SIM and D. C. CARTER, *University Departments of Surgery and Biochemistry, Royal Infirmary, Glasgow*

Blackburn *et al.* (1973) have suggested that isotonic amino acid infusions given without additional energy can, by promoting the use of body fat stores as an energy source, improve N balance after surgery. To examine the effect of these solutions on postoperative energy balance, sixteen patients following major abdominal surgery were given constant intravenous infusions (2 l/d) of either 3·5% isotonic amino acid solution (AA), providing 10 g N/day, or 5% dextrose (D_5W) in addition to their other fluid requirements for the first 4 postoperative days. Six patients received AA and nine received D_5W.

Urine collections (24 h) were made for total N and β-hydroxybutyrate estimation. Indirect calorimetry was carried out on either the third or fourth postoperative day. The accuracy of the calorimeter had been checked by burning commercial butane in the head canopy. The recovery of O_2 and CO_2 was 97·2 and 97·4% respectively and the measured and calculated RQ values for butane were the same (0·619).

	D_5W		AA		
	Mean	SD	Mean	SD	
Total energy expenditure (MJ(kcal)/d)	6·8 (1619)	1·5 (371)	6·8 (1609)	1·2 (284)	NS
Total N excretion (g/d)	8·2	4·0	16·7	2·6	$P<0·001$
Total heat production from protein (%)	12·8	4·0	27·5	3·4	$P<0·001$
Total heat production from fat (%)	62·5	5·3	72·5	3·4	$P<0·001$
Total heat production from glucose (%)	24·7	5·9		0	
Measured RQ	0·737	0·015	0·692	0·014	$P<0·001$

There was no significant difference between the mean N balance of 8·2±4·0 g/d and 6·7±2·6 g/d following D_5W and AA respectively. Using the total O_2 consumption, CO_2 production and N excretion, values for N-free RQ (Kleiber, 1900) were calculated to be 0·72±0·18 and 0·64±0·03 following D_5W and AA respectively. These RQ values are significantly different ($P<0·001$).

From the percentage of fat and glucose contribution to N-free heat production, the predicted N-free RQ values were calculated to be 0·78 following D_5W and 0·71 for AA. The lower than predicted RQ values suggest only partial adaptation to endogenous fat utilization, this being more marked with AA where the measured N-free RQ was 0·64. This, in addition to an increased β-hydroxybutyrate excretion of 3·850±6·0 mmol/d compared with 0·106±0·094 in D_5W is consistent with incomplete oxidation of fat.

This study has failed to demonstrate any improvement in N balance in patients receiving isotonic amino acid infusions and suggests that such infusions are associated with incomplete oxidation of fat and inefficient use of body fat stores.

Blackburn, G. L. (1973). *Ann. Surg.* **177:5**, 588.
Kleiber, M. (1961). In *The Fire of Life an Introduction to Animal Energetics*, p. 85 University of California: Davis.

Effect of 3 d feeding with corn oil supplemented diets on foetal lipid stores in rabbits. By J. P. STAMMERS, M. C. ELPHICK and D. HULL, *Department of Child Health, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH*

The foetal fat stores of the rabbit double in size during the last 3 d of gestation. At term (31 d) adipose tissue represents 5% of body-weight (Hudson & Hull, 1975). The rabbit placenta has high levels of lipoprotein lipase activity (Elphick & Hull, 1977) and it was thought possible that the accumulated foetal lipid might be derived from maternal circulating dietary fat. To test this possibility two groups of rabbits in the twenty-eighth day of pregnancy were fed on either standard laboratory diet (Pilsbury's SG1 390 g fat/kg, 15 g linoleic acid (18:2)/kg) or the same diet enriched with 6–7% corn oil (109 g fat/kg, 58 g linoleic acid/kg).

Feeding was continued for 3 d until term, when delivery was induced with ocytocin. Three foetuses from each doe were killed and liver and brown (BAT) and white (WAT) adipose tissues were weighed and their fat content and triacylglycerol (TG) fatty acid composition measured.

Total mean food intakes over the 3 d were similar, 390 g control, 338 g corn oil fed, but mean fat consumption differed, 12·3 g and 41·7 g respectively. Total body-, BAT, WAT and liver weights of the foetuses were similar in both groups, but fat content of tissues tended to be higher in the corn oil fed group (see Table). The biggest difference, however, was that adipose tissue and liver in the experimental group had nearly double content of linoleic acid compared with controls. Since linoleic acid is an essential fatty acid and cannot be synthetized by the foetus, these results demonstrate that maternal dietary fat is available as a source of foetal stored lipid. These observations lend support to the view that under the influence of placental lipoprotein lipase maternal TG are a source of foetal lipids.

The effect of corn oil feeding on liver and adipose tissue fat contents of foetal rabbits

(Control five does and fifteen foetuses, mean body-weight 49·4 g; corn oil fed six does and seventeen foetuses, mean body-weight 49·8 g)

	Controls				Corn oil fed			
			Fatty acid content (g/100 g)				Fatty acid content (g/100 g)	
Organ	Weight (g)	Total fat (g/100 g)	16:0	18:2	Weight (g)	Total fat (g/100 g)	16:0	18:2
BAT	2·39	42·9	14	9**	2·46	47·8	13	14**
WAT	0·22	39·4	14	8*	0·22	46·8	14	14*
Liver	3·39	11·9***	4	3*	3·42	15·3***	4	6*

Difference from controls (Student's *t* test) *$P<0.001$, **$P<0.01$, ***$P<0.05$.

Elphick, M. C. & Hull, D. (1977). *J. Physiol., Lond.* **273**, 475.
Hudson, D. G. & Hull, D. (1975). *Biol. Neonate* **27**, 71.