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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Eighty-sixth Meeting of the Nutrition Society was held at the Royal Society of Medicine, Wimpole Street, London, on Tuesday, 17 *May* 1983, *when the following papers were read:*

https://doi.org/10.1079/PNS19830061 Published online by Cambridge University Press

Effects of vitamin B_6 deficiency on the nuclear uptake of [^{3}H]testosterone in the prostate of the rat. By DAVID A. BENDER, JEAN-FRANÇOIS BOWDEN, WILLIAM F. COULSON, ELIZABETH K. SYMES and NADA BAAH, *Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 7PN*

Cidlowski & Thanassi (1981) suggested that the metabolically active form of vitamin B_6, pyridoxal phosphate, may have a function in the action of steroid hormones. We have recently shown that there is increased accumulation of [^{3}H]oestradiol in the nuclei of the uterus in vitamin B_6-deficient rats (Holley *et al.* 1983).

We have now extended these studies to the uptake of [^{3}H]testosterone into the prostate of the male rat. Animals were maintained from 6 weeks of age on a vitamin B_6-free diet ((g/kg) maize starch 400, sucrose 200, casein 282, gelatin 60, maize oil 15, mineral salts 41, vitamin mixture 2; 35% of metabolizable energy from protein). Control animals received the same diet supplemented with 5 mg/kg pyridoxine hydrochloride. After 4 weeks each animal received 10 μCi [^{3}H] testosterone intraperitoneally (107 Ci/mmol) and, after 15, 30, 60, 120 and 240 min, eight animals in each group were killed. Prostate glands were dissected out and homogenized in 0·25 M-sucrose and the radioactivities in the total homogenate and in the nuclear and high-speed supernatant fractions were determined.

A considerable amount of vitamin B_6-depletion was achieved (mean ± SE, liver pyridoxal phosphate of 5·9±0·22 nmol/g in deficient animals, 11·4±0·26 nmol/g in controls, $P<0·001$). The accumulation of radioactivity in the whole homogenate and nuclear fraction was significantly higher at 15–60 min in the deficient animals than in controls, with no difference by 240 min. The ratio of radioactivity in the nucleus:high-speed supernatant fraction (an index of nuclear accumulation of the steroid) was maximal in control animals at 15 min, and the half-time of the observed decline in nuclear accumulation was 40 (SE 3·7) min. In vitamin B_6-deficient animals the nucleus:supernatant fraction did not reach a maximum until 60 min after injection of the radioactive steroid, and the half-time of the decline in nuclear radioactivity was significantly longer (421 (SE 60·9) min, $P<0·001$).

These results are in agreement with the suggestion that pyridoxal phosphate may be important in the action of steroid hormones, and suggest that the principal action of the vitamin may be in the turnover of the steroid–receptor complex by the nucleus.

Cidlowski, J. A. & Thanassi, J. W. (1981). *J. Steroid Biochem.* **15**, 11.
Holley, J., Bender, D. A., Coulson, W. F. & Symes, E. K. (1983). *J. Steroid Biochem.* **18**, 161.

A comparison of breast-milk taurine concentrations in vegans and omnivores. By SURINDER K. RANA and T. A. B. SANDERS, *Department of Nutrition, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH*

Human milk contains a substantial amount of taurine, unlike bovine milk and artificial formulas derived from it (Gaull *et al.* 1977). Consequently it has been postulated, mainly on teleological grounds, that the human infant may require dietary taurine. Taurine is only found in food of animal origin, particularly offal and fish (Roe & Weston, 1965), but can also be derived indirectly from dietary cysteine and methionine. However, in vitro studies suggest that the capacity of liver to synthesize taurine from cysteine and methionine is almost as low in man as it is in the cat, which has a requirement for dietary taurine (Rassin *et al.* 1978). Very little is known about the influence of diet on breast-milk taurine concentration. In order to evaluate the contribution made by dietary taurine to breast-milk taurine, we have measured taurine concentrations in mid-stream samples obtained 4–6 weeks post partum from fourteen mothers following a typical omnivore diet, and from fourteen vegan mothers whose diets are devoid of taurine. The results are shown in the Table.

		Breast-milk taurine (μmol/l)		
	n	Mean	SE	Range
Vegans	14	277	28·4	122–529
Omnivores	14	427	37·8	191–683

Statistical significance $P<0.01$.

Despite the absence of taurine from the diets of the vegans, their breast-milk still contained substantial amounts of taurine, which must have been derived from dietary precursors. However, the mean taurine concentration was significantly lower than in the omnivores. If the level of taurine in the breast-milk of the vegans represents optimal synthesis from precursors then it can be inferred that at least one-third of the taurine in the breast-milk of omnivores is derived from preformed dietary taurine.

We are grateful to the Vegetarian Society for a grant.

Gaull, G. E., Rassin, D. K., Raiha, N. C. R. & Heinonen, K. (1977). *J. Paediatr.* **90**, 348.
Rassin, D. K., Sturman, J. A. & Gaull, G. E. (1978). *Early Hum. Devel.* **2**, 1.
Roe, D. A. & Weston, M. O. (1965). *Nature, Lond.* **205**, 287.

Protein intake and its relationship to urinary calcium excretion in the preruminant lamb. By D. M. WALKER and S. AL-ALI, *Department of Animal Husbandry, University of Sydney, Sydney NSW 2006, Australia*

'Protein-induced hypercalciuria' has been reported in man and in the rat (Linkswiler *et al.* 1981), but is unlikely to be common to many species, since the major route for the excretion of calcium in most domestic animals is via the faeces. There are exceptions to this general rule in such species as the rabbit (Cheeke & Amberg, 1973), the horse (Schryver *et al.* 1974), and possibly the pack rat and the hamster (Shirley & Schmidt-Nielsen, 1967), where the urine is an important route for Ca excretion. In some species, e.g. the rat and the dog, although the urine is not the main route for Ca excretion, some variation in urinary Ca content can be achieved by dietary manipulation. The effect of diet on urinary Ca excretion by the preruminant animal has received little attention. It would seem, from the experiments of Hodge (1973), that urinary Ca excretion by the preruminant lamb is less than 1% of Ca intake and is little affected by variation in Ca intake. The effect of protein intake has not been determined previously.

Eighteen male cross-bred lambs, aged 2–5 d at the start of the experiment, were used. The design was a 2×3 factorial in which there were two levels of protein (10 and 30% protein energy as casein) and three levels of Ca at each protein level, equivalent to 50% (low), 100% (medium) and 200% (high) of the Ca concentration in ewes' milk (360 mg Ca/MJ gross energy). The ratio of Ca:P in all diets was 1:1. The intake of gross energy was 1046 kJ/kg body-weight$^{0.73}$ per d. Urine and faeces were collected separately each day and bulked for the last 7 d of the 21 d experimental period.

Protein energy (%) . . .	10			30			
Calcium level . . .	Low	Medium	High	Low	Medium	High	SEM
Number of lambs	3	3	3	3	3	3	
Mean live wt (kg)	6·8	5·9	6·3	6·7	6·8	7·0	0·6
Nitrogen intake (mg/kg per d)	372	394	370	1105	1133	1089	26
Ca intake (mg/kg per d)	112	236	445	111	227	435	9
Faecal Ca (mg/kg per d)	23	97	229	23	65	183	16
Urinary Ca (mg/kg per d)	2·0	1·6	2·0	1·4	0·5	1·4	0·3
Ca balance (mg/kg per d)	87	137	214	87	161	251	20

The results show that urinary Ca excretion in the preruminant lamb is unaffected by either dietary protein concentration, or by the level of dietary Ca, within the range studied.

Cheeke, P. R. & Amberg, J. W. (1973). *J. Anim. Sci.* **37**, 450.
Hodge, R. W. (1973). *Aust. J. agric. Res.* **24**, 237.
Linkswiler, H. M., Zemel, M. B., Hegsted, M. & Schuette, S. (1981). *Fedn Proc. Fedn Am. Socs exp. Biol.* **40**, 2429.
Schryver, H. F., Hintz, H. F. & Lowe, J. E. (1974). *Cornell Vet.* **64**, 493.
Shirley, E. K. & Schmidt-Nielsen, K. (1967). *J. Nutr.* **91**, 496.

Artificial rearing and type of milk substitute affect gut morphology of weanling rats. By J. L. SMART, J. TONKISS and D. N. STEPHENS, *Department of Child Health, The Medical School, Oxford Road, Manchester M13 9PT* and J. EDMOND and N. S. AUESTAD, *Department of Biological Chemistry, ULCA School of Medicine, USA*

Rats were reared artificially without their mothers (Smart *et al.* 1983) from postnatal day 4 or 5 till day 20 or 21, or were reared naturally. Artificially-reared (AR) rats were given either the usual milk substitute for such studies (Messer *et al.* 1969) or a diet resembling rat's milk much more closely in composition (gross composition, g/l: protein 80, carbohydrate 34, fat 110, N. S. Auestad and co-workers, unpublished results). The Auestad 'milk' is derived from a cow's milk base (evaporated milk and skimmed milk powder) which is dialysed to remove lactose and then concentrated (by freezing and thawing) to increase the concentration of protein. Fat, lactose, amino acids, vitamins and minerals are then added in quantities which bring the concentrations of these constituents close to those of rat's milk. Rats given the Messer and Auestad formulae are termed ARM and ARA respectively. There were five experiments. Mother-reared (MR) pups were raised in all experiments, both ARM and ARA pups in two experiments, and only one AR group in three experiments (ARM in one, ARA in two). A total of forty-one MR, eighteen ARM and twenty-six ARA rats were autopsied at 20 or 21 d. Wet weight of empty stomach and length of small intestine (SI) were recorded.

The stomachs of ARA rats were approximately 20% heavier than those of MR rats ($P<0.01$ in each of four experiments). ARM rats had stomachs of normal weight, but their SIs were approximately 17% longer than those of the MR group ($P<0.05$ in each of three experiments). The SIs of ARA rats were less elongated than those of ARM rats ($P<0.05$ in each of two experiments), but were still approximately 9% longer than normal ($P<0.05$ in two out of four experiments).

The greater wet weight of stomach tissue in ARA rats appears to be entirely dependent on diet and independent of the AR process, whereas both of these factors appear to contribute to elongation of SI, in that both AR groups showed this though more markedly in the ARM group. These effects occurred in spite of the fact that the whole body-weight of the AR groups was never greater and sometimes less than that of MR controls. Nor are they readily explicable in terms of differences in osmolarity between the 'milks' (mosmol/l): rat's milk 310, Auestad milk formula 360, Messer milk formula 690.

Messer, M., Thoman, E. B., Terrasa, A. G. & Dallman, P. R. (1969). *J. Nutr.* **98**, 404.
Smart, J. L., Stephens, D. N. & Katz, H. B. (1983). *Br. J. Nutr.* **49**, 497.

Chronic ulcerative disease of the colon in rabbits fed native carageenans.

By S. N. MARCUS, *Gastrointestinal Unit, Walton Hospital, Liverpool L9 1AE* and A. J. MARCUS and J. WATT, *Department of Pathology, University of Liverpool, Liverpool L69 3BX* (Introduced by G. A. J. PITT)

When supplied as drinking fluid, degraded carrageenan, a sulphated polysaccharide of seaweed origin, causes ulcerative disease of the colon in a variety of animal species including mice, rats, guinea-pigs, rabbits and monkeys (Kirsner & Shorter, 1980). We have investigated the long-term effects on the rabbit colon of undegraded or native carrageenans derived from two different species of red seaweed.

Adult male rabbits (New Zealand white, average body-weight 3000 g) were given a standard cube diet supplemented with fresh cabbage. Two groups of twelve animals each received as drinking fluid aqueous solutions (10 g/l) of undegraded (animal grade) carrageenans, obtained commercially and derived from the seaweeds *Chondrus crispus* and *Eucheuma spinosum*. The solutions were freshly prepared each day and supplied *ad lib.* over a period of 6–18 months. The carrageenan solutions were readily accepted as drinking fluid. Control animals received water only as drinking fluid. Clinically the experimental animals showed intermittent diarrhoea associated with the passage of frank or occult blood and mucus; weight gain was significantly reduced in comparison with control animals (*C. crispus* group $P<0.001$; *E. spinosum* group $P<0.001$).

Animals were killed by intravenous Nembutal. The large intestine was emptied of faeces, fixed with formaldehyde and examined by direct and transmitted light. All experimental animals presented features of chronic ulcerative disease of the colon including ulceration, stunting of folds, granular mucosal thickening or stricture formation. The changes were more pronounced in the animals receiving *E. spinosum* carrageenan.

These results indicate that native or undegraded (animal grade) carrageenans are ulcerogenic to the colon when supplied as drinking fluid over a prolonged period. Undegraded carrageenans are added as emulsifiers, stabilizing or gelling agents to many foods including slimming drinks and chemically defined liquid diets. The replacement of carrageenans by a harmless non-sulphated polysaccharide additive would seem logical.

Kirsner, J. B. & Shorter, R. G. (1980). *Inflammatory Bowel Disease*. Philadelphia: Lea and Febiger.

Glucose metabolism in malnutrition: basal glucose turnover and the effect of glucose infusion in the protein-depleted rat. By P. J. CROWE and G. T. ROYLE, *Nuffield Department of Surgery, Oxford* (Introduced by D. H. WILLIAMSON)

Many hospital patients are malnourished and often receive intravenous glucose infusions as nutritional support. However, some studies have shown that malnutrition may cause glucose intolerance (Smith *et al.* 1975; Weinkove *et al.* 1976) and there is doubt about how much glucose can be usefully supplied. It has been shown (Wolfe *et al.* 1978) that tracer methodology using 6-[^{3}H]glucose is a highly accurate method for investigating glucose turnover compared with a glucose tolerance test. In order to study glucose metabolism in malnutrition under carefully controlled conditions, we have used the rat as a model and a 6-[^{3}H]glucose tracer. Animals (250 g) were pair-fed on iso-energetic diets of 140 g (control) and 40 g (malnourished) protein/kg diet for 6 weeks. Studies were performed after a 48 h fast, when basal glucose concentration and turnover were measured and also how an exogenous glucose infusion (39 μmol/kg per min) was removed from the blood.

		Basal glucose (μmol/ml)		Basal insulin (μU/ml)		Basal glucose production (μmol/kg per min)		Glucose clearance post-infusion (ml/kg per min)	
Group	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	7	6·9	0·2	36·6	4·7	61·8	1·7	9·0	0·3
Malnourished	7	5·8$^{\bullet}$	0·5	11·3$^{\bullet\bullet}$	1·3	53·7$^{\bullet\bullet}$	2·3	9·4	0·7

$^{\bullet}P<0·05$, $^{\bullet\bullet}P<0·002$.

Malnourished rats lost body-weight compared with the control rats over the 6 week period ($-1·8\pm7·9\%$ *v.* $38·5\pm10·0\%$ $P<0·001$) and blood glucose and insulin concentrations were lower. The tracer studies show that the low blood glucose concentration was due to decreased endogenous glucose production. Clearance of the exogenous glucose infusion from the blood, however, was equally effective in both control and malnourished animals, as was suppression of endogenous glucose production ($42·9\pm5·3\%$ *v.* $40·3\pm9·9\%$). Insulin concentration increased in both groups (control 59·3 μU/ml, malnourished 20·8 μU/ml) and the percentage increases were not significantly different. Our studies therefore show that protein depletion causes changes in endogenous glucose metabolism but that if provided intravenously, glucose is cleared quite normally from the blood and will effectively reduce the rate of gluconeogenesis from tissue protein. It is likely that glucose has a similar therapeutic effect in clinical malnutrition and that glucose intolerance due to protein depletion is a myth.

Smith, S. R., Edgar, P. J., Pozefsky, T., Chhetri, M. K. & Prout, T. E. (1975). *Metabolism* **24**, 1073.
Weinkove, C., Weinkove, E. A. & Pimstone, B. L. (1976). *Clin. Sci. Mol. Med.* **50**, 153.
Wolfe, R. R., Allsop, J. R. & Burke, J. F. (1978). *Metabolism* **27**, 217.

The effect of continuous and intermittent post-operative nasogastric feeding on oxygen consumption and nitrogen balance. By I. T. CAMPBELL, J. COLE, R. P. MORTON, C. A. RAINE, L. SHAPIRO* and P. STELL, *Royal Liverpool Hospital, Prescot Street, Liverpool L7 8XP* and **St. James's University Hospital, Becket Street, Leeds 7TS*

Because of the abdominal discomfort and diarrhoea associated with enteral nutrition it is now a common practice to use a fine-bore tube to infuse the feed continuously. Feeding patterns are known to affect metabolism and a number of studies have demonstrated a greater weight gain in animals fed intermittently compared with those fed *ad lib.* (Fabry, 1969). The effect of two feeding regimens, a continuous and an intermittent one, on oxygen consumption and nitrogen balance were studied in patients after major surgery. Two groups of male patients, five in each group, comparable with respect to age (60 years), height, weight and nutritional status were fed comparable amounts of Clinifeed-iso (Roussel Laboratories Ltd) via a nasogastric tube for 5 d after major head and neck surgery (see Table). One group was fed continuously, the other group by bolus administration at 2-hourly intervals between 06.00 and 22.00 hours. Resting O_2 consumption ($\dot{V}O_2$) was measured mornings (08.30–09.00 hours) and afternoons (16.30–17.00 hours) for 5 d. Urinary nitrogen excretion (24 h) was measured for 5 d and serum levels of alkaline phosphatase (*EC* 3.1.3.1), alanine aminotransferase (*EC* 2.6.1.2) and glutamyltransferase (*EC* 2.3.2.2), inorganic phosphate and thyroid hormones (T_3, T_4 and rT_3) were estimated daily.

	Energy given (MJ/d)				$\dot{V}O_2$ (ml/min)									
					Pre-op		Day 1				Day 5			
	Day 1		Days 2–5		a.m.		a.m.		p.m.		a.m.		p.m.	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Continuous	4·7	0·5	11·8	0·2	249	8	263	15	311	8	322	17	317	11
Bolus	4·1	0·6	12·0	0·3	252	8	278	6	326	19	254**	11	249**	8

Significant difference between groups: **$P<0.01$.

Oxygen consumption rose in both groups after the operation (see Table). In the continuously-fed patients it remained elevated but in the bolus-fed group it decreased and was significantly lower ($P<0.01$) than the continuously fed patients on the afternoon of day 4, and the morning and afternoon of day 5. Enzyme levels were significantly lower post-operatively ($P<0.05$) in the bolus-fed patients and inorganic phosphate significantly higher ($P<0.05$). There was no difference between the two groups in thyroid hormone concentrations. Cumulative N balance was significantly better in the bolus-fed patients (continuous fed: 5.8 ± 2.7 g, bolus fed 15.9 ± 2.8 g, $P<0.05$).

While continuous nasogastric feeding is almost certainly better than intermittent feeding in terms of avoiding abdominal distension and discomfort, these results indicate that metabolically it may be less efficient.

Fabry, P. (1969). *Feeding Patterns and Nutritional Adaptation.* London: Butterworths.

Methane formation in faunated and ciliate-free cattle. By F. G. WHITELAW, J. MARGARET EADIE, L. A. BRUCE and W. J. SHAND, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It has previously been shown that rumen ciliate protozoa can be established in large numbers in cattle given restricted amounts of an all-concentrate diet and are associated with high proportions of butyric acid and low proportions of propionic acid in the rumen (Whitelaw *et al.* 1972). Since stoichiometric calculations suggested that methane formation might also differ under the two regimens, experiments have been undertaken to compare CH_4 production in the ciliate-free and faunated states.

Twelve Friesian steers with rumen cannulas were used in groups of four in three separate experiments and were given a pelleted diet (850 g barley, 150 g protein supplement/kg diet) in three equal meals at a daily level of 61 g/kg body-weight$^{0.75}$. The ciliate-free condition was maintained by isolation and faunation was achieved by inoculation with a mixed rumen ciliate suspension. As in previous studies, *Entodinium, Epidinium* and *Eremoplastron* spp. became predominant. Measurements of CH_4 production were made over 3–5 d periods in respiration chambers.

			Molar proportions VFA (%)			CH_4 energy (% of gross energy intake)	
Expt	Treatment	*n*	Acetic	Propionic	Butyric	Measured	Calculated‖
1	Ciliate-free	8	45·1	34·8	11·8	—	6·68
	Faunated	8	60·7	11·7	22·2	—	12·12
2a	Ciliate-free	7	46·5	25·5	15·7	—	8·12
2b	Faunated	6	56·6	14·1	22·5	10·48	11·29
3	Ciliate-free	9	45·2	30·9	13·2	6·45	7·19
	Faunated	4	59·1	12·2	22·9	11·45	11·48
Mean differences, SE (pooled estimates)			13·3±2·0	−18·0±1·62	9·0±1·11	5·00±0·73†	4·50±0·42
Significance: *P*			<0·001	<0·001	<0·001	<0·001	<0·001

†Expt 3 only.
‖Stoichiometric calculations.

In each experiment there were highly significant differences between treatments in the molar proportions of the major volatile fatty acids (VFA) (see Table). In Expts 1 and 2a, accurate measurements of CH_4 production were not possible because of calibration errors on the gas analysers. Expts 2b and 3, however, conducted after recalibration of the analysers, showed that CH_4 production differed significantly between treatments ($P<0.001$) and that the observed values corresponded fairly closely with those calculated by stoichiometry.

There were no significant differences between treatments in the apparent digestibilities of dry matter or nitrogen, in the losses of energy in faeces or urine, or in N retention. In Expt 3 the increased loss of energy as CH_4 in the faunated animals resulted in a reduction in the metabolizability of the diet from 0·73 to 0·69 ($P<0.05$).

Whitelaw, F. G., Eadie, J. M., Mann, S. O. & Reid, R. S. (1972). *Br. J. Nutr.* **27**, 425.

Effects of corticosterone acetate on energy balance in mice. By KATHERINE S. GALPIN, R. G. HENDERSON,* W. P. T. JAMES† and P. TRAYHURN, *Dunn Nutrition Unit, Cambridge CB4 1XJ, *Addenbrooke's Hospital, Cambridge CB2 2QQ and †Rowett Research Institute, Aberdeen AB2 9SB*

Administration of corticosteroids to patients is frequently accompanied by a rapid gain in body-weight; similar observations have also been made with experimental animals (Hausberger & Hausberger, 1960). However, the mechanisms responsible for this weight gain are not clear. We have therefore conducted a study to determine whether corticosteroids cause weight gain and the development of obesity solely through the induction of hyperphagia, or whether changes in metabolic efficiency are also involved.

Three groups of male C57BL10ScSn mice, aged 3 months, were housed at 22° and fed on a synthetic diet containing 100 g fat/kg diet. Two of the groups received corticosterone acetate (CA) in the diet at a level adjusted to give a daily intake of 0·35 mg. One group of the CA-treated mice was allowed to feed *ad lib.* while the other group was pair-fed to the *ad lib.* intake of the control animals. Faeces were collected and food intake and body-weight measured daily for 3 weeks. Carcass energy was then determined in all three experimental groups, together with a group of baseline animals, and changes in energy content during the experiment calculated. The energy content of food, faeces and the carcasses was determined using an adiabatic bomb calorimeter. The results are summarized in the Table.

	Controls		CA pair-fed		CA *ad lib.*	
	Mean	SE	Mean	SE	Mean	SE
△ Body-wt (g)	−0·6	0·2	0·1	0·6	5·3***	0·9
Digestible energy intake (kJ)	1240	30	1275	29	1448**	46
△ Carcass-energy (kJ)	9·0	3·0	180·1***	22·3	290·2***	20·5
Gross efficiency (%)	0·8	0·3	14·0***	1·5	19·9***	0·8

n 6; **$P<0.01$, ***$P<0.001$ compared with controls.

The control mice showed only a negligible change in carcass energy and their gross efficiency was almost zero. Treatment with CA resulted in hyperphagia and in a large increase in energy deposition in the *ad lib.*-fed animals. However, there was also a substantial increase in energy deposition in the CA-treated mice which were pair-fed with the controls. The gross efficiency of both groups of CA-treated mice was much greater than for the controls. It is concluded that CA induces excess energy gain in this strain of mice by a combination of hyperphagia and an increased metabolic efficiency. Subsequent studies have indicated that the increase in efficiency is related, at least in part, to a reduction in thermogenesis in brown adipose tissue since decreases in cytochrome oxidase activity and mitochondrial GDP binding have been found in the tissue from the CA-treated animals.

K.S.G. was supported by a grant from the Addenbrooke's Artificial Kidney Patients Association.

Hausberger, F. X. & Hausberger, B. C. (1960). *Am. J. clin. Nutr.* **8**, 671.

Measurement by radioimmunoassay of the mitochondrial uncoupling (GDP binding) protein from brown adipose tissue of lactating mice.

By MARGARET ASHWELL, G. JENNINGS and P. TRAYHURN, *Dunn Nutrition Laboratory, Milton Road, Cambridge CB4 1XJ*

There is increasing evidence from studies on genetically-obese rodents and cafeteria-fed rats that brown adipose tissue (BAT) may play a role in the regulation of energy balance in small mammals. Thermogenesis in BAT is suppressed in the lactating mouse and we have suggested that this is likely to result in a reduction in the maintenance energy expenditure of the lactating animal, leading to a lower than expected energy requirement (Trayhurn *et al.* 1982). The principal evidence for reduced BAT thermogenesis during lactation comes from mitochondrial GDP-binding studies. However, it is not known whether the reduction in GDP binding is due to a decrease in the amount of the uncoupling protein or to a masking of the binding sites. We have now measured the amount of the uncoupling protein in lactating mice by radioimmunoassay using a specific antiserum raised against the protein (Lean *et al.* 1983).

Mice of the 'Aston' variety, aged 2·5–4 months, were used while suckling their first litter. Dams, with five or more pups, were taken at mid-lactation (9–12 d post partum) or at weaning (21 d post partum). Virgin female mice, housed in pairs, were used as controls. [^{3}H]GDP-binding to BAT mitochondria was determined as previously (Trayhurn *et al.* 1982). Uncoupling protein was measured by solid phase radioimmunoassay using a mouse protein standard (Lean *et al.* 1983).

	GDP bound (pmol/mg mitochondrial protein)		Uncoupling protein (μg/mg mitochondrial protein)	
	Mean	SE	Mean	SE
Virgin	352·6	14·5	29·6	3·2
Mid-lactation	74·4***	10·2	3·8***	0·9
Weaning	72·2***	7·8	4·4***	0·9

n 6 or more; ***$P<0.001$ compared with virgin mice.

The Table shows that GDP binding was reduced during lactation, including at the time of weaning. The amount of the uncoupling protein was also decreased in lactation, to a level of less than 15% of that of the virgin animals. Thus the reduced GDP binding to BAT mitochondria of lactating mice is due primarily to a substantial fall in the concentration of the uncoupling protein regulating the mitochondrial proton conductance, rather than to a masking of the binding sites.

Lean, M. E. J., Branch, W. J., James, W. P. T., Jennings, G. & Ashwell, M. (1983). *Bioscience Reports* **3**. (In the Press.)
Trayhurn, P., Douglas, J. B. & McGuckin, M. M. (1982). *Nature, Lond.* **298**, 59.

Factors influencing the occurrence of diet-induced thermogenesis in the rat. By GÁBRIELLE MCKEE, B. DONNE and J. F. ANDREWS, *Department of Physiology, Trinity College, Dublin 2, Irish Republic*

Controversy continues over the putative mechanism of surplus energy dissipation: diet-induced thermogenesis (DIT) (Hervey & Tobin, 1983; Rothwell & Stock, 1983). We have been able to demonstrate DIT in the rat, but our results have shown the effect to be of variable occurrence. This led us to suggest (Andrews & Donne, 1982) that detailed differences in experimental protocol may provide an explanation of the reported differences as to the occurrence/non-occurrence of DIT. But what are these critical differences? We have re-examined our data from this viewpoint and present the following findings.

Rats (male, Wistar) were housed in groups of six from weaning, in open-circuit metabolism chambers, at 30°, and subjected to natural day-length. Animals ranged from 70–100 d of age at the time of study. Oxygen consumption (24 h) was determined in the undisturbed group, as an indirect measure of metabolic rate (Andrews & Mercer, 1973). All animals had *ad lib.* access to a pelleted laboratory rat diet (Odlums (Ireland) Ltd).

1. Sucrose (80 g/l in the drinking water) was required in addition to 'cafeteria' feeding to elicit DIT. For example, in one experiment there was no significant difference between the stock diet/water group ($37{\cdot}5 \pm 1{\cdot}5$) and the cafeteria diet/water group ($37{\cdot}7 \pm 2{\cdot}1$ l O_2/kg body-weight$^{0{\cdot}75}$ per 24 h, mean ± SD of six determinations over 17 d). Addition of sucrose led to a significant increase in the cafeteria diet/sucrose group ($43{\cdot}5 \pm 2{\cdot}8$) over the stock diet/water group ($34{\cdot}9 \pm 2{\cdot}2$ l O_2/kg body-weight$^{0{\cdot}75}$ per 24 h, five determinations over 13 d, $P<0{\cdot}001$). We used a relatively limited variety of foods (twenty-five) in contrast to the fifty used by Rothwell & Stock (1983).

2. Disturbance was shown to influence metabolic rate since moving the chambers during the course of one experiment caused oxygen consumption to fall by 35% in controls (43·9 to 28·5) and by 41% in the cafeteria diet/sucrose group (62·2 to 36·8 l O_2/kg body-weight$^{0{\cdot}75}$ per 24 h). Thus the elevation of metabolism of the cafeteria diet/sucrose group over the stock diet/water group fell from 42% before to 29% 1 d after disturbance.

3. Day-length appeared to influence DIT. In otherwise similar groups of animals, elevation of metabolic rate of a cafeteria group above controls was (l O_2/kg body-weight$^{0{\cdot}75}$ per 24 h): 23 July, 19% (cafeteria 52·3, control 44·0); 1 March, 11% (cafeteria 44·9, control 40·2); 2 November, 0·56% (cafeteria 44·8, control 44·5).

We reiterate our agreement with Rothwell & Stock (1983) that the rat has a mechanism for surplus energy dissipation, but that its stimulation can be influenced by minor differences in experimental protocol. Such differences may provide an explanation of the contradictory findings of the several groups who have investigated this mechanism.

Andrews, J. F. & Donne, B. (1982). *Proc. Nutr. Soc.* **41**, 36A.
Andrews, J. F. & Mercer, J. B. (1973). *J. Physiol., Lond.* **236**, 7P.
Hervey, G. R. & Tobin, G. (1983). *Clin. Sci.* **64**, 7.
Rothwell, N. J. & Stock, M. J. (1983). *Clin. Sci.* **64**, 19.

Longitudinal observations of the food intakes of twenty-one women during pregnancy and lactation. By J. V. G. A. DURNIN, F. M. MCKILLOP, S. GRANT and G. FITZGERALD, *Institute of Physiology, University of Glasgow, Glasgow G12 8QQ*

This paper describes some preliminary findings of the food intakes of the Glasgow section of a multi-centre investigation into the energy requirements of pregnancy and lactation.

Data has been collected on a group of twenty-one women, between 20 and 30 years of age and in their second or third pregnancy. All these women were recruited in the early stages of pregnancy, 6–12 weeks, and were evenly distributed between the manual and non-manual social groups.

Measurements were made every 6 weeks throughout pregnancy and every 4 weeks during lactation. Records of food intake were made during five consecutive days using the individual weighed inventory method.

All of the women had breast-fed their previous infants and were fully intending to breast-feed their new babies: no restriction of their food intakes was consciously observed or mentioned during their pregnancy.

The mean initial weight of this group was $58{\cdot}4 \pm 6{\cdot}5$ kg. The amount of weight gained during pregnancy was variable, the range being 6·0–16·8 kg. On average this represented a 19% increase from their initial recorded weight, the mean weight gain being $10{\cdot}8 \pm 2{\cdot}7$ kg. All the women gave birth to normal, healthy infants with a mean birth weight of $3{\cdot}3 \pm 0{\cdot}4$ kg.

The analysis of the food intakes was done using a food dictionary based on food tables (Paul & Southgate, 1978) with many additional foods included.

The mean values for the energy intakes at various stages of pregnancy and lactation are shown in the Table.

Gestational age (weeks)		kcal/d		(MJ/d)		kcal/kg body-wt per d		kJ/kg body-wt per d	
Mean	Range	Mean	SD	Mean	SD	Mean	SD	Mean	SD
11	(8–13)	2117	426	8·86	1·78	36	8	151	33
16	(14–18)	2167	416	9·07	1·74	37	8	155	33
22	(19–24)	2163	438	9·05	1·83	36	7	151	29
27	(25–30)	2330	460	9·75	1·92	36	8	151	33
32	(31–35)	2138	395	8·95	1·65	33	6	138	25
38	(35–40)	2249	371	9·41	1·55	33	6	138	25
Post partum									
4	(3–5)	2367	547	9·90	2·29	40	9	167	38
8	(6–9)	2212	338	9·29	1·41	37	4	155	17
12	(10–14)	2365	468	9·90	1·96	40	7	167	29

These values do not show any significant increase in energy intake as the pregnancy progresses, although there is a small non-significant increase at 27 weeks. The values obtained during lactation were slightly higher than those of pregnancy but, perhaps because of the sample size and the variability, these were not found to be significantly different.

This research is financed by a grant from the Nestlé Foundation.

Paul, A. A. & Southgate, D. A. T. (1978). *McCance & Widdowson's The Composition of Foods*, 4th revised ed. London: HM Stationery Office.

The use of 'frame' categories in the prediction of fatness. By F. McKay, C. Webster and J. V. G. A. Durnin, *Institute of Physiology, University of Glasgow, Glasgow G12 8QQ*

Assessment of 'fatness' is often made on the basis of the amount of 'overweight' relative to some reference standard. The 'weight for height' tables of the Metropolitan Life Insurance Company of New York (1959) are divided according to body frame categories of small, medium and large. Frame, however, was not defined in any precise way and is clearly not meant to involve any measurement in its use. Other more recent tables also include 'frame' categories, but again lack any quantifiable measurement.

In the present study, 4311 adult men were measured for height, weight and skinfold thicknesses to assess 'fatness', and four skeletal diameters (ulna, tibia, bi-acromial and bi-iliac).

The prediction of both fat-free mass and fatness was not improved by grouping individuals into frame categories. However, introducing ulna diameter into an equation with height and weight was of considerable value in improving the accuracy of these predictions. On the other hand, in this population the easier measurement of calf circumference could be substituted with almost no loss of accuracy.

We conclude that even when skeletal frame is measured, 'frame' categories appear of little value in the prediction of fatness and that there is, therefore, little justification for their use.

This research is financed by a grant from the Ministry of Defence.

Metropolitan Life Insurance Company (1959). *Statist. Bull.* **40**, 1.

Measurement of whole-body protein turnover in neonates with an intragastric infusion of [^{13}C]leucine and with sampling via the urinary leucine pool. By B. DE BENOIS,* Y. ABDULRAZZAK,† O. G. BROOKE,† D. HALLIDAY‖ and D. J. MILLWARD,* **Clinical Nutrition & Metabolism Unit, London School of Hygiene & Tropical Medicine, 4 St. Pancras Way, London NW1 2PE,* and *†Special Care Baby Unit, St. George's Hospital, Tooting, London SW17 0RE,* and *‖Clinical Research Centre, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ*

The measurement of whole-body protein turnover with ^{13}C-labelled (carboxy)leucine is a safe, reliable method which is being increasingly applied in human studies (Halliday & Rennie, 1982). However, measurements of leucine flux usually involve intravenous administration of the isotope and frequent blood sampling. In pre-term infants this may not always be possible and so we have investigated a method involving intragastric infusion of [^{13}C]leucine, with measurement of the enrichment of leucine in the urine as an alternative to plasma. Measurements were made on seven low birth-weight, pre-term infants (1·1–1·8 kg, 29–33 weeks gestational age). Infants were intragastrically fed either human milk (A), or the proprietary formulas Osterfeed (B), Preatamil (Milupa) (C). Infusions generally lasted for 24 h although the plateau enrichment of urinary leucine was reached in all cases within 8 h. The plateau was maintained for at least 48 h according to measurements on one patient. Measurements of plasma and urinary leucine enrichment were similar, the mean ratio was not significantly different from 1 (n 13). Leucine flux was converted into protein synthesis and degradation assuming tissue protein to contain 8% leucine. Rates of protein retention calculated from the leucine flux were similar to those indicated by nitrogen balance measurements. The rates of whole-body protein turnover were not markedly different in any of the groups although more measurements will be required to evaluate any differences between formulas and human milk. This method should enable such studies to be made.

	Intake		Protein synthesis	Protein degradation	Protein retention	
Infusion	(kJ/kg per d)	(mg N/kg per d)	(g/kg per d)	(g/kg per d)	(g/kg per d)	(%N intake)
A	582	461	12·4	10·7	1·7	57
	515	351	11·3	10·1	1·2	57
	565	484	8·1	6·5	1·6	53
B	417	333	11·6	10·1	1·5	74
	517	413	10·9	8·9	2·0	76
C	624	626	13·8	10·3	3·5	90
	569	587	12·3	10·2	2·1	57

Halliday, D. & Rennie, M. J. (1982). *Clin. Sci.* **63**, 485.

Influence of iron, and Fe with riboflavin, on the haematological status of Gambian men. By HILARY J. POWERS and C. J. BATES, *Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ*

Studies with humans and laboratory animals have indicated that riboflavin has some role to play in determining iron economy and haematological status. A study was designed to determine whether an oral Fe supplement could effect an improvement in haematological status in men during the rainy season in rural Gambia, and whether further improvement could be achieved with Fe and riboflavin. Seventy-five men with lower than average haematological status were selected by screening, and allocated to three matched groups, to receive a daily placebo, ferrous sulphate (200 mg), or $FeSO_4$ with riboflavin (5 mg). Blood samples were collected prior to, and at 3 and 6 weeks of supplementation.

Results relating to haematology and Fe status are shown in the Table and were analysed by covariance to eliminate the influence of differences in initial values on the response.

(Change between initial and week 6 measurements)

	Placebo			Iron			Iron + riboflavin		
	Mean	*n*	SD	Mean	*n*	SD	Mean	*n*	SD
EGRAC	−0·27	20	0·47	−0·37	16	0·53	−1·09***	19	0·36
MCV (μ^3)	3·7	20	3·3	6·3*	16	3·0	7·1†	19	4·9
RBC ($\times 10^{-12}$/l)	−0·42	20	0·38	−0·19	16	0·30	−0·12†	19	0·71
Hb (g/100 ml)	−0·20	20	1·08	1·33***	16	1·25	1·26***	18	1·77
PCV (%)	−1·9	20	3·9	1·5**	16	2·7	2·2***	19	5·5
Transferrin saturation (%)	−5·0	20	16·1	8·7†	15	17·6	3·0	18	14·0
Ferritin (ng/ml)	6·4	21	26·2	13·0	18	0·5	21·6*	17	17·0
FEP (ng/ml packed cells)	−19	19	510	−385	15	587	−717	18	949

Value significantly different from placebo: $^{*}P<0·05$, $^{\dagger}P<0·02$, $^{**}P<0·01$, $^{***}P<0·001$.

EGRAC, erythrocyte glutathione reductase activation coefficient; MCV, mean cell volume; RBC, red blood cells; Hb, haemoglobin; PCV, packed cell volume; FEP, free erythrocyte porphyrin.

Both supplements established some improvement over the placebo group. The range of responses within each treatment group, however, tended to be large, and differences in response to Fe + riboflavin compared with the Fe alone appeared small. There are, nevertheless, indications that the Fe + riboflavin group may have utilized the Fe more effectively than in the absence of added riboflavin, leading to a more rapid fall in free erythrocyte porphyrin, a larger increase in mean and packed cell volumes and a more efficient maintenance of RBC numbers. The greater rise in ferritin may represent a more rapid replenishment of Fe stores.

H.J.P. acknowledges a post-doctoral award from F. Hoffmann-La Roche & Co.

Dietary copper and zinc in relation to their distributions in liver fractions.

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Parry & Al-Mukhtar (1980) previously suggested that zinc supplementation in sheep diets influenced plasma-bound copper and, therefore, the Cu in the liver. They also reported that although sheep, a species particularly sensitive to Cu toxicity, had been fed on high-Cu diets supplemented with high concentrations of Zn for many weeks, no apparent symptoms of Cu toxicosis were observed. This paper reports some results of investigations on the distribution of Cu and Zn in liver fractions and liver proteins of sheep fed on high-Cu diets supplemented with high-Zn levels.

Samples of livers from two groups of 6-month-old sheep were analysed for Cu and Zn concentration in whole liver tissue, separated cytosol and particulate fractions, and separated protein Fractions I, II and III. The molecular weights of the proteins in Fractions I, II and III were >75 000, >30 000, and 12 000 respectively. Group A sheep were fed on 700 mg Zn + 6 mg Cu/kg diet, group B sheep were fed on 700 mg Zn + 200 mg Cu/kg diet.

Mean concentration of Cu and Zn in protein fractions for groups A and B

	Group A				Group B			
	Cu (μg/ml)		Zn (μg/ml)		Cu (μg/ml)		Zn (μg/ml)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fraction I	0·15	0·01	0·73	0·02	1·31•••	0·05	0·41	0·04
Fraction II	0·16	0·01	0·27	0·01	0·33	0·01	0·23	0·01
Fraction III	0·52	0·02	0·13	0·01	0·68•	0·02	0·09	0·01

Significance of difference from group A: •$P<0.05$, •••$P<0.001$.

The Cu concentration in the particulate fraction of group B livers was significantly higher ($P<0.001$) than in livers of group A.

Fractionating the cytosol proteins showed that the Cu concentration in Fraction III (metallothionein-like protein) of group B was significantly higher ($P<0.05$) than in Fraction III of group A. The Cu concentration in Fraction I of group B was significantly higher ($P<0.001$) than the Cu concentration in Fraction III of group B and also significantly higher ($P<0.001$) than the Cu concentration in Fraction I of group A.

Parry, W. H. & Al-Mukhtar, F. (1980). *Proc. Nutr. Soc.* **39**, 54A.

Zinc-containing proteins in rat liver. By IAN JOHNSON, *Department of Biochemistry, The London Hospital Medical College, Turner Street, London E1 2AD*

The presence and level of zinc in various tissues of the body has been known for many years. Zn has also been shown to be present in a number of protein fractions in plasma and tissues (Bremner & Marshall, 1974). There is some discussion as to whether Zn-containing proteins act as carriers for the metal. The purpose of this paper is to indicate which Zn-containing proteins may be the most metabolically active.

Liver from five male rats were taken separately, homogenized in 0·01 M-phosphate buffer (pH 7) and centrifuged at 100 000 *g* for 1 h. A portion of the supernatant was placed on to a column containing Sephacryl S200 gel and the proteins present in the supernatant separated and identified by u.v. absorption at 285 nm. The separated fractions from the column were also examined for Zn using atomic absorption spectrophotometry at 213·8 nm.

On the basis that Zn-carrier proteins would preferentially bind the metal compared with other proteins in which Zn may be present, samples of liver homogenate were incubated at 37° in a buffer solution containing added Zn either as zinc chloride or $^{65}ZnCl_2$. The proteins from these incubated homogenates were separated by gel filtration and identified by u.v. absorption peaks at 285 nm. Each fraction of the eluate from the gel filtration was estimated for Zn or ^{65}Zn and the values obtained compared with the u.v. absorption pattern. Zn-carrier proteins showed raised Zn levels following incubation with either $ZnCl_2$ or $^{65}ZnCl_2$.

The findings support earlier reports (Chesters & Will, 1981) that metallothionein (10 000 daltons), albumin (60 000 daltons) and transferrin (85 000 daltons) are Zn-containing proteins in liver, and that another protein of molecular mass 110 000 daltons, distinct from caeruloplasmin, is also involved.

Bremner, I. & Marshall, R. B. (1974). *Br. J. Nutr.* **32**, 283.
Chesters, J. K. & Will, M. (1981). *Br. J. Nutr.* **46**, 111.

The use of lithium as a marker of sodium intake. By CLAUDIA P. SANCHEZ-CASTILLO, *Dunn Clinical Nutrition Centre, Old Addenbrokes Hospital, Trumpington Street, Cambridge CB2 1QE* and W. PHILIP T. JAMES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

There is no simple technique as yet available for estimating the contribution of sodium from various sources to the total intake of individuals within the community. Estimates are at present based on the use of the National Food Survey and food table values for the Na content of foods, together with estimates for the amount of Na added during cooking or at table. The amounts added are not necessarily the same as those ingested. A novel method has, therefore, been developed using lithium-labelled salt to estimate dietary Na derived either from salt added at the table or during cooking.

The method is based on the use of minute quantities of Li, fused as lithium carbonate with sodium chloride, in a fixed ratio at 900°. After grinding and sieving, and with the use of a hygroscopic agent, the new salt behaves on pouring in a similar way to table salt.

Li does not occur in appreciable quantities in most human diets; an epidemiological study in a Cambridgeshire town has shown that the mean (± SE) 24-h excretion of Li is 3·17±1·03 μmol which is less than 1% of the mean Li intake when used as a marker. Normal marker Li intakes are calculated to be 1% of the therapeutic dose. Cooking experiments with various vegetables showed that Li is taken up in parallel with Na by the different plants.

Physiological experiments on eight subjects studied for 8 d and given 250 μmol Li as a single dose showed a recovery of Li of 92±5% over the subsequent 6 d. Continuous Li-labelling studies on ten adults showed that a supplementary dose of 100 mmol Na did not alter Li excretion. These results suggest that this method should prove to be a valuable new tool for estimating the fraction of Na derived from different sources in the diet.