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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Thirtieth Meeting of the Nutrition Society was held at the Royal Society of Medicine, London, on Tuesday, 8 *May* 1979 *when the following papers were read:*

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The in vitro metabolism of 2,6-diaminopimelic acid by rumen micro-organisms. By HILARY A. LANE and J. R. LING, *Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth SY23 3DD*

2,6-Diaminopimelic acid (DAPA) is widely used as a marker of the rumen outflow of bacterial N (Ling & Buttery, 1978) and its decarboxylation by protozoa, to produce lysine, may be of benefit to the host animal (Onodera *et al.* 1974).

Conditions for the in vitro incubation of mixed populations of protozoa isolated from the rumen of sheep were initially studied. The most appropriate (as judged by sustained cilial movement) were the salt solution of Hungate (1942) supplemented with (g/l): rice starch 0·3, ampicillin 1·0, sodium hydrogen carbonate 1·0 and L-cysteine hydrochloride (neutralized) 1·6. Aliquots of 5·0 ml containing about 10^6 protozoa were incubated anaerobically (95% N_2×5% CO_2) at 39°.

Incubations with 0·1 mM-(DL+meso)-2,6-diamino [G-^{3}H] pimelic acid (370 kBq/μmol), purified by ion exchange column chromatography, were continued for up to 8 h, after which the protozoal suspensions were fractionated into pellet (200 *g* for 30 s) and deproteinized (10 g/l picric acid) supernatant. Since isolated rumen protozoa are always contaminated with bacteria, 'protozoa-free' rumen fluid was also incubated with radiolabelled DAPA (Bryant & Robinson, 1961). The bacterial suspensions were similarly fractionated (30 000 *g* for 15 min).

Lysine was separated by ion exchange column chromatography and the amount produced from DAPA (see Table) was estimated by liquid scintillation counting. More lysine was released into the supernatant than was incorporated by the protozoa, whereas the opposite was observed for the bacteria.

(Values refer to approximately 2×10^5 protozoa with 2×10^8 bacteria and 2×10^8 bacteria respectively for the protozoal and bacterial suspensions)

	Protozoal suspensions		Bacterial suspensions	
Incubation time (h)	Hydrolysed pellet	Deproteinized supernatant	Hydrolysed pellet	Deproteinized supernatant
0	0·01	0·04	ND	0·02
1	0·15	0·45	0·03	0·03
2	0·32	1·15	0·05	0·02
4	0·61	3·90	0·08	0·02
8	0·34	8·82	0·16	0·02

ND, not detected.

Tentative estimates of the contributions to duodenal lysine supply by both protozoal and bacterial DAPA decarboxylation would be less than 200 and 40 mg/d respectively in the sheep.

H.A.L. acknowledges receipt of a Science Research Council Studentship.

Bryant, M. P. & Robinson, I. M. (1961). *J. Dairy Sci.* **44**, 1446.
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Ling, J. R. & Buttery, P. J. (1978). *Br. J. Nutr.* **39**, 165.
Onodera, R., Shinjo, T. & Kandatsu, M. (1974). *Agric. biol. Chem.* **38**, 921.

0029-6651/79/3830-330A $00.35

Diet and activity in obese patients in a metabolic ward. By PENELOPE WARWICK and J. S. GARROW, *Medical Research Council, Clinical Research Centre, Watford Road, Harrow HA1 3UJ*

Obesity has been blamed on inactivity (Bloom & Eidex, 1967). However, a restricted diet causes inactivity (Taylor & Keys, 1950) and feelings of lethargy (Young *et al.* 1960). To investigate these relationships fifty-two obese patients, weight 93·9±20·7 kg, were studied in a metabolic ward on a protocol approved by the Hospital Ethical Committee. On admission to the ward patients were weighed, measured and classified as 'bingers' if they admitted a tendency to compulsive eating, and 'non-bingers' if they did not. For the first week all patients received similar diets providing 3·3–4·2 MJ/d, and all kept activity diaries throughout this week. Bingers (n 23) were heavier (109 *v.* 82 kg, $P<0·001$) and fatter (W/H^2 39·7 *v.* 31·5) than non-bingers, and also spent more time standing (min/d; 198 *v.* 152, $P<0·05$). This finding is contrary to the idea that inactivity is proportional to the degree of obesity.

During the second and third week in the ward fourteen patients who had previously received 3·3 MJ/d were restricted to 0·8 MJ/d, while eighteen patients continued at 3·3 MJ/d. In the more severely restricted group of patients time on feet fell from 172 min/d during the first week to 140, and 140 min/d during the second and third weeks ($P<0·05$). Among the eighteen patients who continued on 3·3 MJ/d there was no significant change in time on feet between the first and later weeks.

We conclude that severe dietary restriction reduced spontaneous physical activity in these obese patients.

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Thermogenic effects of diet and exercise. By M. GLEESON, J. F. BROWN and J. J. WARING, *Biology Division, Preston Polytechnic, Preston PR1 2TQ* and M. J. STOCK, *Department of Physiology, Queen Elizabeth College, London W8 7AH*

The thermic responses of animals given a balanced diet is usually regarded as a constant characteristic of the food ingested. However, Miller *et al.* (1967) reported that physical activity performed by human subjects increased the thermic response to food. In subsequent studies of the effect of exercise on dietary thermogenesis both increases (Bray *et al.* 1974) and no differences (Sims, 1976) have been reported.

We have examined the effects of a single meal on energy expenditure at rest and during exercise in normal and exercise-trained male rats and have investigated the possible modifying effect of diet by feeding either a low-fat diet (LF) or a high-fat diet (HF) containing 50 and 300 g corn oil/kg respectively.

Thermic responses in the resting and exercising state

		Postprandial rise (kJ/h)		Thermic response (% BMR)	
	Group	Mean	SE	Mean	SE
Resting	LF Trained	0·67	0·08	10·0	1·2
	LF Sedentary	0·31	0·05	3·9	0·6
	HF Trained	1·00	0·09	13·5	1·2
	HF Sedentary	0·47	0·08	5·4	0·9
Exercising	LF Trained	1·66	0·23	24·7	3·4
	LF Sedentary	2·21	0·56	27·4	6·9
	HF Trained	2·32	0·50	27·4	6·9
	HF Sedentary	4·51	0·78	51·1	8·8

Ingestion of a 120 kJ meal increased oxygen consumption (thermic effect) by 5% in normal rats but this effect was more than doubled in exercise-trained rats. During a standardized treadmill running exercise there was an additional thermic effect which in the normal rats resulted in a sevenfold increase in thermic response compared to resting animals.

These observations confirm that dietary thermogenesis is potentiated by exercise, and furthermore, that exercise-training produces adaptations that result in increased dietary thermogenesis under resting conditions as previously reported by us (Gleeson *et al.* 1978).

Miller, D. S., Mumford, P. & Stock, M. J. (1967). *Am. J. clin. Nutr.* **20**, 1223.
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Sims, E. A. H. (1976). *Clinics in Endocrinology and Metabolism* **5**, 377.
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The effects on respiratory quotient and metabolic rate of glucose, fructose and sucrose in rats. By N. SHARIEF and I. MACDONALD, *Department of Physiology, Guy's Hospital Medical School, London SE1*

Work in rats (Macdonald & Grenby, 1979), baboons (Brook & Noel, 1969) and man (Macdonald & Taylor, 1973) had reported that not all dietary carbohydrates have the same effect on body-weight, when the input is identical as determined by bomb calorimeter. In order to investigate this effect further the metabolic rate (MR) of rats was measured, as was the respiratory quotient (RQ) following the ingestion of glucose, fructose, both monosaccharides together and sucrose, with water alone as control.

Rats (body-weight 164–267 g) were fasted overnight following daytime controlled feeding. One mg of carbohydrate/g body-weight in 2 ml water was given by gastric tube under light ether anaesthesia. The amounts of oxygen and carbon dioxide in the expired air were measured, and from the values obtained, the RQ and MR (Weir, 1949) determined. Six animals were used for each sugar.

The results showed that the RQ following the ingestion of fructose was greater than that after glucose and when these two monosaccharides were given together at the same total dose, the RQ was as predicted from the two monosaccharides given separately. After sucrose ingestion the RQ was significantly greater than that after the component monosaccharides given together, and the value after sucrose exceeded 1·0. These results are similar to those found in man (Higgins, 1916).

The MR for the first 105 min after intubation of sucrose was significantly greater than glucose, fructose or a mixture of the two. However, the MR following sucrose ingestion tended to return to the fasting value sooner than the other carbohydrates tested. The MR following the glucose and fructose mixture was greater after 105 min than expected from the values obtained from the two monosaccharides given separately.

Brook, M. & Noel, P. (1969). *Nature, Lond.* **222**, 562.
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Macdonald, I. & Grenby, T. H. (1979). *Proc. Nut. Soc.* **38**, 30A.
Macdonald, I. & Taylor, J. (1973). *Guy's Hosp. Rep.* **122**, 155.
Weir, J. B. V. (1949). *J. Physiol., Lond.* **109**, 1.

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Influence of dietary fluoride and molybdenum on the skeleton of rats. By J. QUARTERMAN, J. N. MORRISON, E. MORRISON and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Results obtained by Burns & Allcroft (1964) during a survey of fluorosis in cattle suggested that a concurrent copper deficiency induced by a high molybdenum intake might exacerbate the dental and skeletal lesions resulting from the ingestion of fluoride. Suspicions that Mo either directly, or by antagonizing Cu utilization, may modify and exacerbate the symptoms of fluorosis in man also arise from recent studies of the aetiology of the bone disease 'genu valgum' in Andra Pradesh, India (Krishnamachari & Krishnaswarmy, 1974).

We have examined the influence of variations in the dietary content of Cu and Mo upon the composition and mechanical properties of the bones of rats offered diets omitting or containing fluoride. Groups of ten weanling rats were offered for 3 months a semi-synthetic diet which contained 2·5 mg Cu/kg with, or without, sodium fluoride (100 mg F/kg) and Mo (3 mg Mo/kg) as ammonium tetrathiomolybdate. Molybdenum was given in this form because of preliminary indications that the thiomolybdate (TM) ion or its derivatives may be involved in the induction of Cu deficiency in animals exposed to high dietary intakes of molybdenum and sulphur. The breaking stress (BS) and Young's modulus (YM) of freshly dissected femurs and tibias were measured with an Instron 1195 (High Wycombe, Bucks).

The effects of copper, fluoride and thiomolybdate (TM) on bone breaking stress (BS, N mm^{-2}), Young's modulus (YM, N mm^{-2}), hydroxyproline (μg/g WW), Cu (μg/g WW) and F (μg/g dry fat-free bone)

	Tibia		Femur		Femur	Femur	Tibia
Dietary supplement	BS	YM	BS	YM	Hyp	Cu	F
—	488	716	315	413	46	4·6	210
F	394	672	280	368	41	3·7	4420
F×TM	405	563	255	320	37	7·3	4950
Mean SE	14	32	10	21	1·4	0·2	110

Addition of F to the diet reduced BS and YM of tibia and femur and the hydroxyproline and copper content of femur (see Table). The hydroxyproline and copper contents of femur were significantly depressed in animals receiving TM alone, but the small quantity of Mo added in this form had no significant effect upon the physical characteristics of bone. When F and TM were given simultaneously, the YM of tibia and femur and the BS of femur were reduced to a significantly greater extent than when F was given alone ($P<0·01$, $<0·05$, and $<0·05$ respectively). The simultaneous administration of F and TM increased the content of both F and Cu in bone.

We are grateful to Mr P. Bowker, Department of Medical Physics, Aberdeen University Medical School, for advice and assistance.

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The effect of dietary carbohydrates and other diet constituents on nephrocalcinosis in the rat. By J. A. OXLEY and K. R. BRUCKDORFER, *Department of Biochemistry, Royal Free Hospital School of Medicine, 8 Hunter St., London WC1N 1BP*, G. EDWARDS, *RHM Research Limited, Lord Rank Research Centre, High Wycombe, Bucks HP12 3QR* and J. YUDKIN, *Queen Elizabeth College, Campden Hill Road, London W8 7AH*

Renal calcification is commonly found in older rats, especially when they are fed on semi-synthetic diets. The calcification occurs even more commonly when the diets contain sucrose rather than starch (Kang *et al.* 1979) and these pathological changes can be observed within a few weeks (J. A. Oxley, unpublished results). We have now compared the effects of four different carbohydrates.

Four groups each of eight male Sprague-Dawley rats were fed for 3 months on diets with 500 (g/kg) sucrose, maize starch, glucose or fructose, together with 200 (g/kg) butter, 190 (g/kg) casein and vitamins and mineral mixtures (Kang *et al.* 1979). The rats were killed by exsanguination, and the kidneys removed, decapsulated and prepared for histological examination and for mineral analysis by atomic absorption spectrophotometry.

Rats given sucrose had more nephrocalcinosis than those fed on the other carbohydrates. This was reflected by a higher concentration of kidney calcium in the rats fed on sucrose; there was no difference in the concentrations of magnesium (see Table). Rats given stock diet had no histological signs of nephrocalcinosis, and the calcium concentration was $0{\cdot}33 \pm 0{\cdot}03$ mg/g kidney. In similar experiments the nephrocalcinosis induced by dietary sucrose was considerably reduced by the addition to the diet of methionine (2g/kg diet), or by the increase in dietary magnesium from 0·6 g to 2·0 g/kg diet. Rats fed on fructose developed no more nephrocalcinosis than did those fed on starch, indicating that this effect of sucrose does not depend on its fructose moiety.

A similar experiment with female rats confirmed the common finding of a greater prevalence of nephrocalcinosis than with male rats; as with male rats, dietary sucrose enhanced this prevalence.

The effects of diet on nephrocalcinosis in the rat are therefore complex. The nature of the dietary carbohydrate is important, but the effects are considerably modified by other dietary components.

J.A.O thanks the SRC and RHM Research Ltd. for a CASE award.

Calcium and magnesium content of the kidneys of rats given diets containing different carbohydrates (500 *g/kg diet*) *for* 3 *months*

(Mean values with their standard errors for eight rats. Statistical significance of difference from the starch group in parentheses (Student's *t* test))

	Sucrose		Starch		Glucose		Fructose	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Kidney wt g/kg body-wt	3·1	0·14	2·7	0·14	2·1	0·11	2·4	0·11
Calcium mg/g kidney	4·44 ($P<0{\cdot}001$)	0·47	0·73	0·12	3·29	0·91	1·58	0·28
Magnesium mg/g kidney	0·36	0·02	0·34	0·02	0·43	0·06	0·43	0·04

Kang, S. S., Price, R. G., Yudkin, J., Worcester, N. A. & Bruckdorfer, K. R. (1979). *Br. J. Nutr.* **41**, 65.

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Reduction of blood lipids by guar crispbread. By D. J. A. JENKINS, D. REYNOLDS, BRENDA SLAVIN, A. R. LEEDS, ALEXANDRA L. WALLER and L. M. JEPSON, *Department of Regius Professor of Medicine, Radcliffe Infirmary and University Laboratory of Physiology, Oxford, and MRC Unit and Departments of Cardiology and Chemical Pathology, Central Middlesex Hospital, London*

Of those unabsorbable plant polysaccharides which may be classified as dietary fibre, the gums, gels and mucilages have consistently been shown to be the most potent in lowering serum cholesterol level. However, the major problem which has prevented their use in treating clinical states where raised blood lipids are found is the difficulty of incorporating these viscous materials into palatable foods. With the development of a palatable guar crispbread containing 1 g guar, 2·4 g starch and 2·6 g gluten/slice, it has now been possible to assess the longer-term effects on blood lipids of one of the most effective of this class of substances. In addition, it has allowed comparison with other modes of administering guar and with other hypocholesterolaemic agents.

Eleven type II or IV hyperlipidaemic patients (four men, seven women, 56±3 years, 116±6% desirable weight) took an average of 13 g guar in crispbread form over 2 to 8 week periods. Eight weeks treatment (seven patients) reduced total serum cholesterol by 13% ($P<0.02$), the fall being in the LDL fraction (16%, $P<0.02$) while HDL cholesterol was unchanged. A 13% non-significant reduction was also seen in serum triglyceride while there was a small but significant fall in body-weight (1·5 kg, $P<0.002$). Comparison of blood lipid changes over 2 week periods showed guar crispbread to be as effective as guar given in hydrated (fruit juice, skim-milk and soup, eight patients) or semi-hydrated form (50% in soup and 50% incorporated into ordinary bread, four patients).

Mean changes (%) in blood lipids and body-weight over 2 weeks treatment with hydrated and semi-hydrated guar and cholestyramine compared with guar crispbread

	Pts	Cholesterol			Triglyceride	Body-weight	Guar (g/d)
		Total	LDL	HDL			
Hydrated	8	−8*	−11*	4	−0·6	−0·7	11
Crispbread		−9†	−10	0·3	−9	−0·5	12
Semi-hydrated	4	−3	−5	−3	3	0	11
Crispbread		−11	−12	−4	−27	−0·4	14
Cholestyramine	5	−8	−13	4	9	0·4	–
Crispbread		−11	−12	4	−14	−1·4	13

*$P<0.05$. †$P<0.02$.

In addition, total serum cholesterol was lowered significantly (11%, $P<0.05$) in five patients where cholestyramine was ineffective. Due to its acceptability guar crispbread is likely to prove a useful cholesterol-lowering agent.

We thank Mr D. Heath and Mr P. Lees of Speywood Laboratories Ltd, Bingham, Nottingham, for development and provision of guar crispbread. D.J.A.J. is in receipt of a grant from the British Diabetic Association.

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Reduced dietary-induced thermogenesis in obese subjects before and after weight loss. By P. S. SHETTY, R. T. JUNG and W. P. T. JAMES, *MRC Dunn Clinical Nutrition Centre, Addenbrooke's Hospital, Cambridge*

A smaller thermogenic response to a meal in obese subjects has been suggested as the factor responsible for their propensity to store fat but early attempts to assess dietary-induced thermogenesis (DIT) gave variable results. A recent careful study with oral glucose, however, showed a reduced response in obese women (Pittet *et al.* 1976). This study was designed to test whether subnormal thermogenesis occurred with a mixed diet and whether the difference could be an intrinsic defect rather than a response to the obese state as such.

Five lean women with no family history of obesity and an ability to eat *ad lib.* without weight gain were studied; their mean weight was 53·7±3·7 kg (mean±SEM), 9·6% below the ideal body-weight (IBW) for height. Five obese patients of similar age with a family history of obesity and weighing 88·5±7·7 kg (+54·2% IBW) were studied on a weight maintenance regime (HED) providing 167 kJ (40 kcals) /kg IBW. Another five previously obese patients ('post-obese') had reduced their weight to 64·3±2·7 kg (+9·1% IBW) and were studied after maintaining their weight for three months. Resting metabolic rate (RMR) was measured by the ventilated hood technique after an overnight fast under thermoneutral conditions. A standard liquid meal of Carnation 'Build-up' in milk was then given to provide 41 kJ (9·8 kcal)/kg IBW, with the protein : carbohydrate : fat energy ratios of 1·5 : 3·3 : 2·2 and the RMR was measured for 2 h thereafter. The lean group had a greater peak rise in RMR of 0·74 kJ/min than the obese (0·48 kJ/min) and post-obese patients (0·44 kJ/min). The integrated response in RMR over 2 h following the meal was also significantly greater in the lean than in the obese ($P<0·02$) and post-obese group ($P<0·01$). To assess the effects of short-term energy restriction on DIT two obese subjects were studied on the HED regime and after 3 weeks on a 38·5 kJ (9·2 kcal)/kg IBW intake. The postprandial response was similar on both diets. In normal weight controls and obese subjects administration of an acaloric drink had no effect on RMR over the same duration of time.

Plasma glucose increased whereas plasma free fatty acids, triglycerides and glycerol tended to fall after the meal in all groups. Plasma concentrations of noradrenaline increased more in the obese and post-obese groups than in the lean subjects. This different catecholamine response and the subnormal DIT in the obese patients may indicate differences in autonomic catecholamine metabolism in obese and lean subjects consistent with the observed differences in the thermogenic capacity of lean and obese individuals (Jung *et al.* 1979).

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The viability and glucagon responsiveness of isolated hepatocytes from adult sheep. By I. A. DONALDSON, M. A. LOMAX and C. I. POGSON, *Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ*

We report here a modification of the caudate lobe perfusion of Clark *et al.* (1976). which gives an improved yield of cells over the slicing technique of Ash & Pogson (1977) and which, with the reduced time of collagenase digestion, provides cells with sensitivity to glucagon.

Within 1 min of slaughter, the portal branch supplying the caudate lobe was cannulated; the lobe was perfused with oxygenated Ca^{2+}-free Krebs-Henseleit buffer (KHB) containing 0·5mM-EGTA. The lobe (7–25g) was excised and rapidly connected to a recirculating perfusion apparatus. The washing procedure was continued at a flow-rate of 80 ml/min (37°) for 5 min. Flow was then switched to a second reservoir containing 200 ml of complete KHB and 80 mg collagenase (Boehringer) for 30–40 min. The capsule was then opened, the cells dispersed and filtered through a nylon 150 μm mesh filter, washed twice with 40 ml KHB plus 2% albumin and centrifuged at 40 g for 2 min.

Total cellular ATP, measured with a specific firefly luciferase assay, was greater than 6 nmol /mg dry wt (see Table), and remained around 80% of this value after 90 min incubation with 10 mM-propionate. The percentage of cells able to exclude 10 mM-succinate was routinely >80%, providing further evidence of plasma membrane integrity. Endogenous glucose production was three times greater in cells isolated from fed than from 4 d fasted animals; propionate increased release to a greater extent in cells from fed sheep.

(Mean values with their standard errors. No. of animals in parentheses)

	ATP (nmol/mg)		Succinate (%)		Glucose production (nmol/mg per h)					
					Endogenous		10 mM-propionate			
							Total		Net	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Fed	6·74	0·336	83	1·88	176	24·7	419	33·9	243	18·9
	(12)		(8)		(12)		(12)		(12)	
4 d fasted	6·01	0·342	82	5·76	60	31·9**	182	34·1***	122	10·0***
	(8)		(4)		(8)		(8)		(8)	

Statistical significance of difference between fed and starved animals, **$P<0.01$, ***$P<0.001$.

In 'fed' cells, 10^{-6}M-glucagon caused a two to fourfold stimulation of cyclic AMP levels, from a basal value of 1·6–3·8 pmol /mg dry wt; cyclic AMP levels remained elevated for 30 min thereafter. Half-maximal response was seen between 5×10^{-8} and 5×10^{-9}M-glucagon..

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Clark, M. G., Filsell, O. H. & Jarrett, I. G. (1976). *Biochem. J.* **156**, 671.

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Palmitate metabolism by isolated hepatocytes from fed and fasted sheep.

By M. A. LOMAX, I. A. DONALDSON and C. I. POGSON, *Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ*

An increase in the hepatic output of ketone bodies, accompanied by an increase in the hepatic uptake of free fatty acids occurs in the fasted sheep (Katz & Bergman, 1969). A study was undertaken to examine the relative rates of oxidation and esterification of palmitate and the effects of carnitine and propionate thereon in hepatocytes from fed and 4 d fasted sheep.

Hepatocytes were prepared as described by Donaldson *et al.* (1979). The cells (approximately 5 mg dry wt) were incubated for 90 min at 37° in 2 ml of Krebs-Henseleit buffer containing 2% defatted bovine serum albumin, and, where indicated, 1 mM-[1-^{14}C] palmitate, 1 mM-L-carnitine and 10 mM- propionate. The rates of ketone body release, of palmitate esterification, and of oxidation to $^{14}CO_2$ were found to be linear up to 90 min of incubation.

The rate of ketogenesis was higher in fasted sheep cells. Carnitine stimulated ketogenesis only in hepatocytes from fed sheep. Propionate inhibited ketone body release and stimulated esterification of palmitate in cells from both fed and starved sheep. Palmitate oxidation to $^{14}CO_2$ was significantly stimulated in cells from fed animals only, when both propionate and carnitine were present. It is concluded that fasting promotes the oxidation of palmitate to ketone bodies and that this difference may be related to intracellular carnitine availability. The antiketogenic action of propionate appears to be related to an increase in the rate of esterification of palmitate rather than to an increase in the rate of oxidation of palmitate to CO_2.

(Mean values with their standard errors. No. of animals in parentheses)

	Palmitate (nmol/mg dry wt per h)		Palmitate+ propionate (nmol/mg dry wt per h)		Palmitate+ carnitine (nmol/mg dry wt per h)		Palmitate+propionate+carnitine (nmol/mg dry wt per h)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Ketogenesis:								
Fed	32·6	4·1(11)	6·0	1·7(5)	87·4	8·3(11)	55·1	6·8(4)
Fasted	86·2	3·7(6)	29·7	7·6(3)	92·3	5·0(6)	73·8	10·4(3)
Palmitate esterified:								
Fed	20·6	2·7(7)	46·3	1·3(4)	17·6	2·4(7)	49·4	4·2(3)
Fasted	23·6	3·1(6)	53·8	10·9(3)	22·2	2·6(6)	51·0	11·8(3)
Palmitate oxidized to $^{14}CO^2$:								
Fed	4·3	0·5(10)	4·4	0·9(5)	4·9	0·3(10)	12·6	1·6(4)
Fasted	4·4	0·8(6)	7·6	1·6(3)	5·2	0·8(6)	7·3	1·7(3)

This work was supported by the Agricultural Research Council and Roussel Uclaf S.A.

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Effect of insulin treatment on protein turnover in adult diabetics. By E. C. ALBERTSE, P. J. GARLICK, V. M. PAIN, P. J. REEDS*, P. J. WATKINS* and J. C. WATERLOW, *Department of Human Nutrition, London School of Hygiene & Tropical Medicine, London WC1*, and **Diabetic Department, King's College Hospital, London SE5*

Preliminary observations (Waterlow *et al.* 1977) of whole body protein turnover in uncontrolled diabetic patients indicated low synthesis rates which increased with insulin treatment. With a further six subjects, however, we have failed to substantiate this effect of insulin. Male patients with uncontrolled diabetes, despite treatment with oral hypoglycaemic agents, were admitted to the diabetic ward to commence insulin therapy. Before and after initiation of the insulin regime, rates of whole body synthesis and breakdown were measured by administration of a single dose of [^{15}N] glycine, followed by measurement of ^{15}N abundance in urinary ammonia (Waterlow *et al.* 1978). Before treatment synthesis and breakdown rates were within the normal range (see Table, Expt. 1), although the patients showed negative N-balance, hyperglycaemia and extreme glycosuria. After 4–5 d of insulin therapy there appeared to be a fall in both synthesis and breakdown. The change in whole body breakdown was greater, but both changes were statistically insignificant ($P<0.05$), despite an improvement in N-balance in most patients. Similar trends were observed in two further subjects (Expt. 2) on a modified dietary protocol. The rate of excretion of 3-methyl histidine in the urine, which may be used as a measurement of breakdown of muscle protein, was unaltered by insulin therapy.

Protein synthesis and breakdown (g protein/kg per d) and nitrogen balance (gN/d) in diabetic patients before and after insulin treatment

	Pre-insulin			Post-insulin		
	Synthesis	Breakdown	N-Balance	Synthesis	Breakdown	N-Balance
Expt. 1						
1	3·1	3·0	+0·9	2·4	2·3	+1·5
2	3·0	3·8	−7·8	3·2	3·0	+2·1
3	4·6	4·6	−0·1	2·8	2·3	+0·5
4	4·5	4·6	−1·5	4·2	4·3	−1·4
Mean difference (post-insulin—pre-insulin)				−0·65	−1·03	+2·8
SEM				±0·43	±0·44	±2·4
Expt. 2						
5	6·2	6·3	−6·8	6·0	6·3	−6·6
6	6·9	6·4	−2·5	5·4	4·9	+0·5

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Polyamine excretion by trenbolone acetate treated rats. By J. T. PEARSON and P. J. BUTTERY, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicester LE12 5RD*

Trenbolone acetate (3-oxo-17β hydroxy-4,9,11, estratriene acetate) is a valuable commercial anabolic agent.

Thirty female specific pathogen-free rats of the Wistar strain were housed in individual metabolism crates with continuous access to both food and water. They were injected daily for 14 d subcutaneously via the neck skinfold with either 100 or 1000 μg of testosterone or trenbolone acetate (TBA) dissolved in 0·1 ml corn oil/100 g body-weight. Control rats were injected with corn oil alone. During days 11–14 of the trial, 24 h urine samples were collected over acid (to minimize bacterial contamination) and pooled.

The urine was prepared according to the method of Henningson *et al.* (1976). Chromatographic separation was carried out at 58° on a 130×10 mm column of Aminex A5 resin (Bio-Rad Laboratories, Bromley, Kent), eluted with sodium citrate buffers, pH 6·16, IM (with respect to Na^+ 0–70 min, pH 4·68, 2·4M, 70–250 min, pH 4·68, 3·05M, 250–430 min, flow-rate 0·5 ml/min. Peaks co-chromatographing with standards of histamine, putrescine, cadaverine, and spermidine were detected in the urine of both treated and untreated rats (Table).

(Mean values for six animals/treatment)

	Histamine (μ mol/d)	Putrescine (μ mol/d)	Cadaverine (μ mol/d)	Spermidine (μ mol/d)	Wt gain (g/14d)
Control	6·7	4·6	1·7	·74	48·6
Testosterone 100 μg/100 g body-wt per d	9·0NS	4·9 NS	1·8 NS	1·5***	63·7**
Testosterone 1000 μg/100 g body-wt per d	13·3**	6·17***	3·5***	1·0NS	49·0NS
TBA 100 μg/100 g body-wt per d	13·9***	4·4NS	2·2NS	2·0***	64·9**
TBA 1000 μg/100 g body-wt per d	19·2***	4·2NS	3·0**	2·1***	61·7*
Pooled SEM	1·31	0·531	0·271	0·144	3·70

NS, not significant. Statistical significance of difference from controls (Duncan's multiple range), $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

These increases in cadaverine and spermidine excretion following TBA treatment are consistent with the observed changes in the muscle intracellular concentrations of lysine and arginine (Vernon & Buttery, 1978). Polyamines are intimately involved with RNA metabolism (Cohen, 1971) and indeed TBA has been shown to affect muscle RNA concentration (Vernon & Buttery, 1978).

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Protective effect of ω3-polyunsaturated fatty acids in chick nutritional encephalomalacia. By P. BUDOWSKI*, N. FLINT and M. A. CRAWFORD, *Nuffield Laboratories of Comparative Medicine, The Zoological Society of London*

Nutritional encephalomalacia (NE) has been observed in young chicks receiving diets deficient in vitamin E and containing a source of linoleic acid, but when α-linolenic acid was substituted for linoleic acid, no NE occurred (Dam *et al.* 1958; Century & Horwitt, 1959; Machlin & Gordon, 1960). It has also been reported that α-linolenic acid and cod-liver oil fatty acids reduced the incidence of NE in chicks receiving linoleic acid (Century & Horwitt, 1959).

We have examined the effects of fatty acids from safflower-seed oil, linseed oil, and cod-liver oil on the incidence of NE. The fatty acid methyl esters from these oils were given to 1-d-old chicks as 80 g/kg of a diet based on extracted soya-bean meal, glucose monohydrate, minerals and vitamins, except vitamin E. The diets also contained 50 mg of the antioxidant BHT/kg.

At the end of the experiment, nearly all chicks in the safflower group had NE. On the other hand, not a single case of NE was observed in the linseed group, and the linseed esters considerably reduced the incidence of NE when added to a diet containing safflower esters. Cod liver esters induced NE in some of the chicks, but also exerted some protective effect on chicks receiving safflower esters.

The dietary treatments profoundly affected the fatty acid composition of brain ethanolamine phosphoglycerides. At the end of the experiment, the proportions of total ω6 fatty acids in cerebella in the linseed and cod liver groups were one-third and one-sixth, respectively, of the value found in the safflower group, while total ω3 fatty acids were twice as high as in the safflower group. The approximate ratios of ω3 to ω6 fatty acids were 0·7, 3·5 and 6·0 for the safflower, linseed and cod liver groups, respectively. The corresponding ratios in the cerebra were slightly lower than in the cerebella, but otherwise, the fatty acid patterns were similar in these two regions of the brain.

The protective effects of linseed and cod-liver fatty acids against NE could be related to the increased ω3 to ω6 fatty acid ratios in brain lipids, but the relation is not a simple one, as linseed fatty acids had a greater protective effect than cod-liver fatty acids but produced less drastic changes in fatty acid patterns. Nor is the special role of the cerebellum as the target organ in NE clear from the present results. The analytical approach should be extended to other lipid fractions and the possibility should be considered that the cyclooxygenase system plays an active part in the aetiology of NE. It may be that the specific effect on the cerebellum is associated with the development events occurring during this period in the cerebellum but not in the cerebrum.

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*On sabbatical leave from the Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel.

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The determination of the oxidation rate of ^{14}C-labelled substrates in man by measurement of plasma total $^{14}CO_2$. By M. B. CLAGUE and M. J. KEIR (Introduced by M. WHITEHOUSE), *Departments of Surgery and Medical Physics, Royal Victoria Infirmary, Newcastle upon Tyne*

The oxidation rate of most ^{14}C-labelled substrates is determined by measurement of $^{14}CO_2$ expiration using a mouthpiece or some form of vented hood. In man this technique may be poorly tolerated especially after surgery. If the size of the rapidly turning-over bicarbonate pool relative to body-weight (Winchell *et al.* 1970) and CO_2 turnover at rest remain fairly constant, total $^{14}CO_2$ in an aliquot of plasma should be proportional to [^{14}C]bicarbonate entry into that pool and oxidation of the ^{14}C-labelled substrate could be determined without the need to collect expired air.

Sodium [^{14}C]bicarbonate in saline was given as a predetermined priming dose followed by a constant rate infusion to thirteen patients preoperatively, six of these patients receiving a second infusion 1–3 d post-operatively. The infusion rate (μCi/h) could be accurately determined from the activity of the infusate (disintigrations/min per g) and subsequent calibration of the needle, syringe and pump (g/h). Permission was obtained from the Isotope Advisory Panel and the local Ethical Committee for use of up to 5 μCi on two separate occasions in patients over the age of 45 years. Three samples of venous blood were taken at a time when $^{14}CO_2$ had been shown to have established a plateau in expired air (60, 75 and 90 min), and the total $^{14}CO_2$ activity in plasma (disintegrations/min per ml) determined by liquid scintillation counting and corrected for varying pool size between individuals (disintegrations/min per ml per 70 kg body-weight).

This was plotted against the infusion rate of $NaH^{14}CO_3$ (μCi/h) and subjected to linear regression analysis. A good correlation was obtained (r 0·878, P=0·001; 82 disintegrations/min per ml per 70 kg body-weight = 1 μCi $H^{14}CO_3$ input/h). There were no significant differences between the pre- and post-operative results (P 0·65).

This technique appears to be valid and has been used by ourselves in subsequent metabolic studies. It is more acceptable to patients than techniques requiring collections of expired air and does not require separate determination of the label retained within the more slowly turning-over bicarbonate pool.

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Synthesis of arachidonic acid by foetal rat brain. By T. A. B. SANDERS and D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, Campden Hill, London W8 7AH*

Arachidonic acid (20:4ω6) is the predominant polyunsaturated fatty acid in foetal rat brain, where it is associated with neuronal membrane phosphoglycerides. Although arachidonic acid cannot be synthesized *de novo*, linoleic acid (18:2ω6) can be converted to arachidonic acid by liver microsomes in adult rats by the following sequence (Sprecher, 1977),

$$18{:}2\omega6 \rightarrow 18{:}3\omega6 \rightarrow 20{:}3\omega6 \rightarrow 20{:}4\omega6$$

In the rat, neuronal multiplication occurs mainly in utero, and is almost complete by the third day *post partum* (Dobbing, 1970). Whether the brain requires a preformed supply of arachidonic acid during this period is not, however, known. We decided, therefore, to examine the capacity of foetal brain to convert linoleic acid to arachidonic acid.

Rat foetuses, obtained on day 21 of pregnancy, were injected intracranially with 1μCi [1-C^{14}] linoleic acid (60mCi/mmol), as the potassium salt, in 10 μl buffered isotonic saline, pH 7·4. They were kept alive for 2 h before being killed. Fatty acid methyl esters, prepared from total brain lipid extracts, were analysed by radio-gas-liquid chromatography.

(Mean values with their standard errors for four analyses)

	18:2ω6		18:3ω6		20:3ω6		20:4ω6	
Fatty acids	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Distribution of methyl esters by weight (mg/g)	7	0·9	Trace		4	1·3	117	2·5
Distribution of radioactivity among methyl esters (counts/10^3 counts)	656	21	48	5	73	11	111	9

A considerable proportion of the recovered activity was associated with the desaturation products of linoleic acid, notably with arachidonic acid. These findings imply that the foetal rat brain may not require a preformed supply of arachidonic acid.

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Riboflavin levels in the diet and breast milk of vegans and omnivores. By JANE HUGHES and T. A. B. SANDERS, *Department of Nutrition, Queen Elizabeth College, Campden Hill, London W8 7AH*

The levels of water-soluble vitamins in human milk are known to be influenced by maternal dietary intake (Kon & Mawson, 1950). Vegans generally have lower intakes of riboflavin than omnivores owing to the absence of dairy products from their diet (Ellis & Mumford, 1967). We decided, therefore, to look for differences in the concentrations of riboflavin in breast milk between vegans and omnivores.

Vegan mothers were contacted through the Vegan Society and omnivores through the National Childbirth Trust. Mothers were visited at home 4–12 weeks *post partum*. They were asked to record their food intakes for 3 d and on the third day to provide three separate samples of fore-milk for analysis.

(Mean values, with ranges in parentheses, for six vegans and nine omnivores)

	Riboflavin	
	Diet (mg/d)	Breast milk (μg/l)
Vegans	1·33 (0·73–2·05)	310 (236–362)
Omnivores	2·45 (1·65–4·45)	393 (310–480)
Statistical significance of difference	$P<0·05$	$P<0·05$

The vegan subjects had been on a vegan diet on average for 7 years (range 2·5–16). All the subjects were successfully lactating. The mean dietary riboflavin intake of the vegans was lower than that of the omnivores, both in absolute terms and when energy intake was taken into account; the vegan diets provided a mean of 0·163 mg/MJ (range 0·108–0·292) compared with 0·235 mg/MJ (0·135–0·460) in those of the omnivores. In two vegans and one omnivore, riboflavin intakes failed to meet the recommended intake of 0·143 mg/MJ (0·6 mg/1000 Kcal) (FAO/WHO, 1974) but in none of the subjects was the intake indicative of deficiency. The mean milk riboflavin concentration was lower in the vegans than in the omnivores but similar to the values reported for pooled milk samples from five different areas of the United Kingdom (Department of Health and Social Security, 1977).

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