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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Ninety-third Meeting of the Nutrition Society was held in the Physiology and Anatomy Lecture Theatres, Guy's Hospital Medical School, London, on Thursday, 15 December 1983, when the following papers were read:

Wholemeal guar bread: acceptability and efficacy combined. A study of blood glucose, plasma insulin and palatability in normal subjects. By VICTORIA BURLEY, ANTHONY R. LEEDS, PETER R. ELLIS and DAVID B. PETERSON, *Department of Nutrition and Food Science, Queen Elizabeth College, London W8 7AH*

The potentially beneficial effects of guar gum on blood glucose control in diabetic patients have been demonstrated in a number of centres, but there have been problems with its administration due to its highly unpalatable nature. The objective of this study was to determine the optimum level of guar incorporation into wholemeal (wheat) bread to achieve reduced insulinaemia while retaining palatability.

After overnight fasts, fifteen healthy subjects took meals of wholemeal bread or bread supplemented with guar at three different concentrations (50, 100 and 150 g guar/kg flour) with sufficient water to make the total meal weight up to 200 g. To eliminate the problem associated with starch reduction due to dilution by guar, the subjects were required to eat three control wholemeal bread meals with starch levels corresponding to those of the three guar breads. Two meals of wholemeal control bread containing 48 g starch were also given on separate occasions. Meal order was randomized. Venous blood samples were taken preprandially and at 30 and 60 min after commencement of the meal and were analysed for glucose by a glucose-oxidase method and for insulin by radioimmunoassay. On a separate occasion the acceptability of the bread was studied using a hedonic score method.

No significant differences in blood glucose were observed between guar and the control breads except for the 150 g guar/kg bread at 30 min ($P<0.001$). Insulin levels were different at 30 min (see Table) but not at 60 min.

Change in plasma insulin (mU/l) from fasting values at 30 min (n 15)

Starch (g/meal) ...	48		41		35·5		30	
Meal type ...	48 g starch Control	48 g starch Control	41 g starch Control	50 g guar/kg flour	35·5 g starch Control	100 g guar/kg flour	30 g starch Control	150 g guar/kg flour
Mean	24	28	21	14	22	11	20	8
SEM	5·1	5·1	4·3	2·5	3·1	2·1	4·7	1·6
						$P<0.005$		$P<0.05$

Mean hedonic scores of 50 and 100 g guar/kg breads were below a neutral score of 5 indicating acceptability whereas 150 g guar/kg bread had a mean hedonic score above 5 indicating lack of acceptability.

Wholemeal guar bread at the 100 g guar/kg flour level was judged palatable and was efficacious, thus it may be a useful alternative to white guar bread in future studies on diabetic patients.

Lactose production, milk volume and lactose synthetase (*EC* 2.4.1.22) activity in rats fed on diets of varying protein adequacy. By Y. K. C. Mansaray and R. Grimble, *Nutrition Department, Southampton University, Southampton SO9 3TU*

A diet containing a low concentration of good quality protein reduces lactose synthetase (*EC* 2.4.1.22; LS) activity in lactating rats and pup growth (Mansaray & Grimble, 1983). This observation was investigated further. Pregnant Wistar rats fed on a standard laboratory chow were caged separately. Litter sizes were adjusted to nine and the dams assigned to one of six dietary groups. The diets contained high or low concentrations of good or poor quality proteins, in the form of milk protein supplemented with L-methionine, or cereal protein. Milk protein concentrations were 200, 100 or 60 g/kg diet. Cereal protein concentrations were 200 and 100 g/kg. All diets were isoenergetic. Cereal diets caused a reduced appetite. A group receiving 200 g milk protein/kg diet was pair-fed with the 200 g cereal protein/kg diet group. All other groups were fed *ad lib.* Milk volumes were measured on the 14th day of lactation by the method of Rath & Thenen (1979). On the 15th day dams were anaesthetized with Nembutal. Milk (1 ml) was removed from the inguinal glands on one side; the glands on the opposite side were removed and frozen in liquid nitrogen. LS activity was measured in whole tissue homogenates and the effect of added bovine α-lactalbumin (10 mg/g tissue; LA) on LS activity determined (Mansaray & Grimble, 1983). Milk was assayed enzymically for lactose.

	Milk protein				Cereal protein	
Dietary protein (g/kg)	200	100	60	200§	200	100
Number of animals	6	6	5	5	6	6
Food intake (g/dam per 13 d)	541	488	314*	312*	310*	300*
LS activity‡	28·9	24·2	14·5*	21·1	11·6*†	11·0*†
Stimulation by LA (%)	8·9	13·5	56·0*	4·8	57·0*	66·1*
Total lactose (g/dam per d)	1·77	1·39	0·54*†	0·83*	0·44*†	0·50*†
Lactose concentration (g/l)	33·4	30·8	23·4*	24·1*	22·6*	21·8*
Milk volume (ml/dam per d)	52·8	44·5*	23·0*†	34·7*	19·6*†	23·0*†
Pup growth (g/pup per d)	1·92	1·57*	0·65*†	1·23*	0·64*†	0·59*†

Values significantly different from *ad lib.* fed 200 g milk protein/kg diet group: *P<0·05.
Values significantly less than pair-fed 200 g milk protein/kg diet group: †P<0·05.
‡Activity expressed as nmol × 10⁻² product formed/30 min per g tissue at 37°.
§Pair-fed with 200 g cereal protein/kg diet group.

Lactose production decreased in all groups which had reduced food intakes, irrespective of protein quantity or quality. Comparison of values from groups given the 60 g milk protein/kg and 200 g cereal protein/kg diets, with the pair-fed group, suggests that inadequate protein quantity and quality also decrease lactose production. The decrease was due to reduced milk volume and concentration. The reductions in lactose production were correlated with LS activity (*r* 0·794, *P*<0·001) suggesting that the diets affected lactose production via this enzyme. Exogenous α-lactalbumin was able to largely correct the reduction in LS activity suggesting that the α-lactalbumin part of the LS complex is particularly sensitive to dietary inadequacy.

Mansaray, Y. K. C. & Grimble, R. (1983). *Proceedings of the Nutrition Society* **42,** 140A.
Rath, E. A. & Thenen, S. N. (1979). *Journal of Nutrition* **100,** 840–847.

The effect of $n6$ and $n3$ polyunsaturated fatty acids on testicular development in the rat. By W. M. F. LEAT, CHRISTINE A. NORTHROP, K. DAVIDSON and F. A. HARRISON, *AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

Rats given linolenic acid ($18:3n3$) as a sole source of essential fatty acids (EFA) develop testicular degeneration whereas those given only linoleic acid ($18:2n6$) have normal testicular development (Leat *et al.* 1983). Experiments to compare the effect on testicular development of other fatty acids of the $n6$ and $n3$ series, namely arachidonic ($20:n6$), docosatetraenoic ($22:4n6$) and docosahexaenoic ($22:6n3$), are now reported.

Five rats aged 6 weeks and five rats aged 4 weeks, born to two dams reared on $18:3n3$ as the sole source of EFA, were used in two experiments respectively. All rats were fed on an EFA-deficient diet, and one animal from each group received daily supplements of the methyl esters of one of the pure fatty acids: (i) $18:2n6$ (ii) $20:4n6$ (iii) $22:4n6$ or (iv) $22:6n3$, with one control animal (v) in each group receiving no supplement of fatty acid. In Expt 1, left orchidectomy was carried out on each animal under general anaesthesia after 5 weeks of supplementation. The rats in both experiments were killed by anaesthetic overdose after 9 weeks of treatment.

Body-weight (g) and testis weight (g) of rats fed on n6 or n3 fatty acids for 9 weeks

	Expt 1		Expt 2		
Supplement (g)	Body-wt	Right testis	Body-wt	Right testis	Left testis
(i) $18:2n6$ (4)	332	0·550	316	1·061	1·119
(ii) $20:4n6$ (2)	341	0·875	349	1·301	1·403
(iii) $22:4n6$ (2)	320	0·720	369	1·069	1·080
(iv) $22:6n3$ (2)	277	0·401	276	0·397	0·473
(v) Nil (0)	225	0·473	222	0·459	0·445

The $n6$ fatty acids stimulated the growth and development of the testis when compared with the deficient rats, and the greatest response occurred with $20:4n6$ (see Table). Spermatozoa were detected in the lumina of the tubules and in the epididymides of the animals fed on $n6$ acids; the major polyunsaturated fatty acid of the testicular phospholipids was $22:5n6$ (14–19%). Testicular degeneration similar to that produced with $18:3n3$ supplements (Leat *et al.* 1983) occurred in rats given $22:6n3$. The percentage of $22:5n6$ in the testicular phospholipids of these rats was low ($<3\%$) and no spermatozoa could be detected in the testes or epididymides.

Leat, W. M. F., Northrop, C. A., Harrison, F. A. & Cox, R. W. (1983). *Quarterly Journal of Experimental Physiology* **68**, 221–231.

Changes in lipid composition of grass during ensiling with or without added fat or oil. By W. STEELE and R. C. NOBLE, *Hannah Research Institute, Ayr KA6 5HL*

Under normal indoor feeding, a dairy cow given a silage-based diet receives an intake of 700–1400 g lipid/d. Because of the physical constraints imposed by forage-based diets, high-yielding dairy cows receiving such rations may not be able to consume sufficient energy to meet the energy requirements of their potential milk production. Thus attempts have been made to increase the energy density of the diet by inclusions of fats and oils.

Although the composition of grass lipids has been extensively studied (Kates, 1970; Harwood, 1980), only a few investigations have been devoted to the changes which occur in the lipids of grass during ensiling or other storage processes (Lough & Anderson, 1973). The present experiment, therefore, has been designed to study the changes in lipid composition which occur during ensiling of (a) high-quality grass, (b) medium-quality grass, (c) medium-quality grass plus (1·85% w/w) tallow, and (d) medium-quality grass plus (1·85% w/w) groundnut oil.

The major fatty acid-containing fractions in the grass were phospholipids (PL), digalactosyl diglycerides (DGDG), monogalactosyl diglycerides (MGDG) and triglycerides (TG). During storage there was considerable hydrolysis of these fractions, in particular the DGDG fraction, which by the end of the ensiling period was present in trace amounts only. There was a concomitant increase in the amount of free fatty acids (FFA) as these fractions decreased.

The addition of tallow and groundnut oil to the grass at the time of ensiling, as expected, increased considerably the TG content of the silage. During ensiling of the grass with added lipid, hydrolysis of lipid fractions occurred similar to that observed with the unsupplemented grass, but included hydrolysis of the added TG from the tallow and groundnut oil.

During ensiling of the unsupplemented grass, linoleic acid (which was the major fatty acid present in the DGDG and MGDG fractions) underwent extensive release with a corresponding increase in its concentration in the FFA fraction. In the silage supplemented with tallow, there was considerable release of stearic and oleic acids from the added TG with resultant large increases in the concentrations of these two acids in the FFA fraction and a corresponding decrease in the proportions of linoleic and linolenic acids, compared with the unsupplemented silage. With the addition of groundnut oil, hydrolysis during ensiling led to marked increases in the concentrations of oleic and linoleic acids in the FFA fractions.

No apparent interconversion of fatty acids were observed in any of the silages.

Harwood, J. L. (1980). In *The Biochemistry of Plants,* vol. 4. *Lipids: Structure and Function,* p. 1 [P. K. Stumpf, editor]. New York: Academic Press.

Kates, M. (1970). *Advances in Lipid Research* **8**, 225–226.

Lough, A. K. & Anderson, L. J. (1973). *Proceedings of the Nutrition Society* **32**, 61A.

Unique lipid patterns associated with the bile of the domestic fowl (*Gallus domesticus*). By R. C. NOBLE and K. CONNOR, *Hannah Research Institute, Ayr KA6 5HL*

In mammalian species there is an established pattern for biliary lipid composition. Phospholipid and free cholesterol account for virtually all the lipid (Portman *et al.* 1975). The function of the bile as a vehicle for the excretion of these lipids is well documented (Portman *et al.* 1975; Myant, 1981). Gall bladder bile of the domestic fowl, from embryo to maturity, possesses a quite unique pattern (see Table) entirely unlike that of animal species so far studied. In bile from the embryonic chicken, phospholipid was less than half of the total lipid present and there were substantial amounts of cholesteryl ester and triglyceride. The cholesteryl ester, the proportion of which increased significantly over the last week of incubation, showed a very high level of oleic acid. By contrast, in both laying and non-laying hens, about 60% of the total biliary lipid was accounted for by phospholipid accompanied by an exceedingly high level, one-quarter of the total lipid present, of triglyceride; low levels only of cholesteryl esters were present.

The lipid composition (weight % of total lipid) of gall bladder bile from the chick embryo, immature and mature chicken, rabbit and sheep

(Values are means, with standard errors, of four observations)

Age ...	Chick embryo				Chicken				Rabbit		Sheep	
	13 d		19 d		7 weeks		36 weeks					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Cholesteryl ester	16·9	1·25	29·6	0·13	2·42	0·82	4·96	1·13	Trace		Trace	
Triglyceride	18·9	1·04	12·1	1·93	26·2	0·03	28·2	3·86	Trace		Trace	
Free fatty acid	Trace		Trace		2·04	0·14	Trace		Trace		Trace	
Free cholesterol	17·3	0·52	18·1	0·34	5·95	0·32	5·04	0·96	12·3	0·28	5·97	0·43
Phospholipid	46·9	1·78	40·3	2·30	63·4	3·04	61·87	4·52	87·5	1·01	93·9	0·58

The livers of the embryonic and mature birds also display unique patterns. The liver lipids of the embryo contained exceptionally high levels (>70% of total lipid) of cholesteryl oleate (Noble & Moore, 1964, 1966). The high levels of cholesteryl ester in the bile of the embryo may represent, therefore, a mechanism through which hepatic accumulation of cholesteryl ester is reduced. Similarly the presence of substantial amounts of triglyceride in the bile of the mature bird may enable control of the hepatic level of triglyceride, particularly in the laying bird.

Myant, N. B. (1981). *The Biology of Cholesterol and Related Steroids*. London: William Heinemann Medical Books Ltd.

Noble, R. C. & Moore, J. H. (1964). *Canadian Journal of Biochemistry* **42**, 1729–1741.

Noble, R. C. & Moore, J. H. (1966). In *Physiology of the Domestic Fowl*, pp. 87–102 [C. Horton-Smith and E. C. Amoroso, editors]. Edinburgh: Oliver and Boyd Ltd.

Portman, O. W., Osuga, T. & Tanaka, N. (1975). *Advances in Lipid Research* **13**, 135–194.

Fractional rates of protein synthesis in zinc-depleted rats. By G. LIVESEY and S. SOUTHON, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA*

Some of the well-recognized adverse effects of dietary zinc deficiency, for example, poor and erratic food intakes, poor growth and poor food utilization, might be secondary to changes in intestinal function. We are therefore investigating the effects of Zn deficiency on the intestine. Decreased rates of protein synthesis have been implicated in the growth retardation observed in many animal species as a result of dietary Zn depletion (Prasad & Oberleas, 1973). This study was undertaken to discover the effect of Zn depletion on the fractional rates of protein synthesis (FRPS) in jejunal and ileal mucosa of rats in vivo. The FRPS in liver was also measured.

Immature Wistar rats were divided into three groups of ten animals. Group 1 received a semi-synthetic diet containing only 2 mg Zn/kg diet. Groups 2 and 3 were pair-fed and *ad lib.*-fed, respectively, with a similar diet containing 60 mg Zn/kg. After 28 d, FRPS values were measured using an intraperitoneal flooding dose of L-[4-^{3}H]phenylalanine as described by Reeds *et al.* (1982). Five animals from each group were killed at 5- and 15-min intervals. For comparison, FRPS values were determined in a fourth group of rats fed *ad lib.* with a stock diet (45 mg Zn/kg).

Rats fed on the low-Zn diet exhibited classical symptoms of Zn deficiency, that is erratic and reduced food intake, poor growth, reduced food utilization and lesions. FRPS values, expressed as %/d, were similar for liver in all groups of animals and agree with earlier findings of a lack of effect in this tissue in Zn-depleted rats (Williams & Chesters, 1970). There was no evidence of reduced rates of protein synthesis in the mucosal tissues from the Zn-depleted animals when compared with pair-fed controls.

FRPS (%/d) (four to five rats per group)

Group	Jejunal mucosa		Ileal mucosa		Liver	
	Mean	SEM	Mean	SEM	Mean	SEM
1. Zn-depleted	83	6	74	8	70	4
2. Pair-fed control	71	4	65	6	59	7
3. *Ad lib.*-fed control	79*	5	55**	4	61	5
4. Stock-fed control	105	6	107	4	60	4

Significantly different from stock-fed control group: *$P<0.01$, **$P<0.001$.

An unexpected observation is the low rates of protein synthesis in the mucosal tissues of rats fed on the semi-synthetic diet (groups 1–3) by comparison with those fed on the stock diet (group 4). The possibility that this is a response to differences in the dietary fibre content of the two diets is being investigated.

Prasad, A. S. & Oberleas, D. (1973). *Journal of Laboratory and Clinical Medicine* **82**, 461–466.

Reeds, P. J., Haggarty, P., Wahle, W. J. & Fletcher, J. M. (1982). *Biochemical Journal* **204**, 393–398.

Williams, R. B. & Chesters, J. K. (1970). *British Journal of Nutrition* **24**, 1053–1059.

A role for zinc in essential fatty acid metabolism. By H. P. FIELD and J. KELLEHER, *Department of Medicine, Clinical Sciences Building, St. James's University Hospital, Leeds LS9 7TF*

In animals and man, zinc and essential fatty acid (EFA) deficiencies show similar pathologies. A biochemical role for Zn in EFA metabolism has been described (Bettger *et al.* 1979; Cunnane, 1982). Various groups of patients at risk of Zn depletion exist. These include the institutionalized elderly, children or pregnant women on poor diets, patients with cancer, Crohn's disease, cystic fibrosis, coeliac disease or liver disease and patients receiving parenteral feeding. Abnormal EFA levels have been demonstrated in similar groups of patients. It is not known whether the abnormal levels of Zn and EFA are interrelated in these patients.

We have used the model of experimental Zn deficiency in male weanling rats to assess the effects of dietary Zn depletion on tissue profiles of EFA and EFA metabolism.

Essential fatty acid content (% of total fatty acids) of tissue lipids from zinc-deficient and control rats

Fatty acid ...	Plasma phospholipid					Liver phospholipid					Plasma total lipid				
	18·2		20·4			18·2		20·4			18·2		20·4		
Dietary group	Mean	SE	Mean	SE	n	Mean	SE	Mean	SE	n	Mean	SE	Mean	SE	n
Zn-deficient	10·35	0·35	27·83	1·33	6	9·77	0·66	30·65	1·21	7	10·67	0·39	37·53	1·43	6
Pair-fed controls	8·67	0·27	33·20	0·60	6	9·23	0·22	35·06	0·66	7	8·97	0·19	38·00	1·59	6

Two groups of seven rats were given synthetic diets for 7 weeks (Table). Blood and tissue levels of phospholipid fatty acids were then determined. Zn deficiency caused a significant increase in the linoleic acid content of plasma phospholipids ($P<0.01$), plasma total lipid ($P<0.005$) and liver phospholipid ($P<0.05$) (Table). Zn deficiency caused a significant decrease in the level of arachidonic acid in plasma and liver phospholipid ($P<0.005$, $P<0.01$ respectively). These results suggest that the synthesis of arachidonic acid from linoleic acid was impaired in Zn deficiency. In a further group of animals the effect of Zn deficiency on the activity of the two desaturase enzymes ($\triangle 5$ and $\triangle 6$) responsible for arachidonic acid synthesis from linoleic acid was studied in liver microsomes. The level of $\triangle 5$ enzyme activity in the Zn-deficient rats (mean 0·112 (SE 0·012) nmol converted/min per mg protein, $n5$) was significantly less ($P<0.05$) than in the control animals (mean 0·162 (SE 0·012) $n5$). These results suggest that Zn deficiency results in a decreased synthesis of microsomal arachidonic acid from linoleic acid and this may explain the quantitative abnormalities seen in essential fatty acids in various tissues in Zn deficiency.

Bettger, W. J., Reeves, P. G., Mascatelli, E. A., Reynolds, G. & O'Dell, B. L. (1979). *Journal of Nutrition* **109**, 480–488.
Cunnane, S. C. (1982). *Progress in Lipid Research* **21**, 73–90.

Effects of energy restriction on baboons given low-protein diets. By P. G.
Lunn and B. A. Baker, *Dunn Nutritional Laboratory, Downham's Lane,
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Recent investigations (Lunn & Austin, 1983*a,b*) have shown that rats given low-protein diets only develop hypoalbuminaemia when their energy (E) consumption is in excess, relative to protein (P) intake. A similar experiment has now been performed using primates.

Hypoalbuminaemia was generated in three young baboons by allowing them free access to a diet with a P:E value of 0·0300 for 3 months. Following this period, the animals were allowed to feed *ad lib.* for a further 3 weeks during which time they were weighed daily and their food consumption measured. On the last day of the third week a blood sample was taken. For the next 3 weeks, food intake was restricted to 90% of *ad lib.* consumption but the P:E value of the diet was raised to 0·0333 so that protein intake remained unchanged. A further blood sample was taken at the end of this period. These manipulations were repeated such that the baboons received sequentially, for 3-week periods, 80, 70, 60, 50, then 60, 70, 80, 90, 100% of their *ad lib.* food consumption, but at each step protein intake was kept constant by adjustment of the P:E value of the diet. The Table shows some of the results from these animals.

Dietary restriction (%) ...	*Ad lib.*		90		80		70		60		50	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
P:E value	0·0300	—	0·0333	—	0·0375	—	0·0429	—	0·0500	—	0·0600	—
Growth (g/week)	45	20	−15	5	43	10	16	7	38	5	12	10
Plasma albumin (g/l)	4·39	0·21	4·57	0·21	4·61	0·16	4·87	0·09	5·16	0·04	5·42	0·12
Plasma triiodothyronine (ng/ml)	2·61	0·05	2·61	0·06	2·53	0·11	2·28	0·11	2·26	0·31	1·98	0·05

Growth rates did not alter significantly during the experiment. Plasma albumin concentration increased with progressive food restriction, reaching control values when energy intake was lowest. Plasma total triiodothyronine was elevated in animals fed *ad lib.* but fell to control levels when food intake was restricted to 50%.

It was concluded that when offered diets of low P:E value baboons, like rats, consume more energy than is physiologically required and that the metabolic response to this energy excess and not to low-protein intake per se results in the appearance of hypoalbuminaemia.

Lunn, P. G. & Austin, S. (1983*a*). *British Journal of Nutrition* **49**, 9–16.
Lunn, P. G. & Austin, S. (1983*b*). *Journal of Nutrition* **113**, 1791–1802.

Methionine to cystine conversion in cattle. By P. J. BUTTERY, CHRISTINE ESSEX, A. N. FOULDS and J. B. SOAR, *Department of Applied Biochemistry and Food Science, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

In many instances there is clear evidence that methionine supply to the duodenum is limiting the growth of beef cattle. Methionine can give rise to cysteine but the proportion of methionine that is used for cysteine synthesis in cattle is not known. Friesian–Hereford cross steers were given a sugar-beet–urea diet (18.2 gN/kg diet) and $[^{35}S]$L-methionine infused for 8 h into one jugular vein. The specific activity of methionine and cystine in the plasma obtained from the other jugular vein was determined by preparative ion-exchange chromatography using the separation system of Jeppson & Karlsson (1972). Three animals (mean weight 177 (SE 12·0) kg) were used and the mean flux through the plasma pool was 5.37 (SE 0·528) mmol methionine/h (equivalent to 21·9 g protein/d per kg body-weight$^{0.75}$). At plateau the cystine-S had a specific activity of 6·7 (SE 1·75) % that of methionine. In a separate experiment, four animals (mean weight 182 (SE 14·2) kg) were infused with $[^{35}S]$cystine and the flux through the plasma pool was 6.59 (SE 1·045) mmol cystine-S/h (equivalent to 46·9 g protein/d per kg body-weight$^{0.75}$). We do not have an explanation for this unexpectedly high flux rate. From these results we conclude that 1·3 g/d of cystine originates from methionine. Such conclusions of course assume that plasma cystine rapidly equilibrates with other pools of free cysteine and cystine. From a separate series of experiments we estimate that the uptake of cystine from the duodenum to be 5 g/d and that of methionine to be 6·75 g/d. The proportion (by weight) of the methionine uptake that is converted to cyst(e)ine would therefore be $((1.3 \times$ molecular weight (MW) methionine/MW cysteine)/methionine uptake) $= ((1.3 \times 149/121)/6.75) = 0.24$.

Jeppson, J. O. & Karlsson, I. M. (1972). *Journal of Chromatography* **72**, 93–103.

Potato crisps in the diet of laboratory rats: effects on body-weight, lipids and dental health. By T. H. GRENBY and M. G. SALDANHA, *Department of Oral Medicine and Pathology, Guy's Hospital, London SE1 9RT*

Changing dietary habits have led to increased consumption of snack foods including potato crisps, but the nutritional consequences of these changes have not been fully explored. The high-fat content of potato crisps (as much as 380 g/kg) has given cause for concern, and they have recently been cited as harmful to the teeth.

As part of a project to investigate these effects, four matched groups of fifteen caries-active Osborne-Mendel rats were fed, for 35 d from weaning, on regimens with two separate components which were available *ad lib.* at the same time: (1) A 'basal' mixture of (g/kg): white flour 365, skim-milk powder 592·5, liver powder 37·5, vitamin, mineral and essential fatty acid supplement 5. (2) Comminuted crisps in three varieties: ready-salted (RS), salt & vinegar (SV) or cheese & onion (CO). The fourth (control) group received a mixture of (g/kg) sucrose 435 and white flour 565.

The energy values of all items of the diet were measured by a bomb calorimeter. Records were kept of food and water intakes and weight gains. At the end of day 35 the mandibular molar teeth were examined for dental plaque and caries. Total body fat was determined by solvent extraction.

Weight gain and total body fat deposits in relation to energy value of food eaten

Diet	Weight gain (mg/kJ food)		Body fat (mg/kJ food)	
	Males	Females	Males	Females
Crisps				
RS	14·8	9·6	1·8	0·9
SV	14·9	11·6	1·5	1·3
CO	16·1	12·7	1·8	1·4
Sucrose/flour	14·3	10·7	1·7	1·4

The main findings were: (i) The rats on the crisps regimens drank on average 55% more water than those on the sucrose/flour regimen. (ii) Compared with the three crisps regimens, the rats on the control regimen ate less of the basal dietary component 1 and more of component 2 (sucrose/flour *v.* crisps), i.e. ≈75% more by weight but only 30% more in terms of energy content. (iii) On the basis that each gram of adipose tissue stores about 31 kJ, the proportion of the total energy intake stored as fat averaged 5·3% in the males and 4·0% in the females. There was no evidence that the crisps regimens were more lipogenic than the sucrose/flour regimen. (iv) Only the sucrose/flour regimen proved to be highly cariogenic, but dental caries attack was significantly greater on the cheese & onion than on the other two kinds of crisps.

The influence of glucose on the plasma galactose response to galactose in the rat, guinea-pig and mouse. By CELIA A. WILLIAMS and ALISON M. OWENS, *Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH*

In man, peroral or intravenous glucose can reduce the serum galactose response to galactose (Williams *et al.* 1983). It has been reported that the rat is dissimilar to man with respect to galactose metabolism (Newstead, 1979).

Galactose (0·5 mg/g body-weight (BW)) was given alone or with glucose (0·5 mg/g BW) to twelve rats (male, Tuck Wistar, ≈90 g), twenty-four mice (male, C.D.I., ≈50 g) and six guinea-pigs (male, Dunkin Hartley, ≈200 g). The test meals were made up in 4 ml water/kg BW and administered via an oro-gastric tube after an overnight fast.

Blood samples were taken from the rats and guinea-pigs before and at 30 and 60 min after administration of the test meal. Blood samples were obtained from four mice by cardiac puncture at each sampling time. Plasma was analysed for galactose and glucose.

Administration of galactose alone produced an increase in the plasma galactose concentration in all animals. Plasma glucose levels increased in the guinea-pigs and mice but not in the rats after the galactose test meal.

Administration of glucose with the galactose did not significantly alter the plasma galactose response of the rat whereas it increased the plasma galactose response of the mice significantly at 30 min ($P<0·01$). Addition of glucose to the galactose test meal administered to the guinea-pigs significantly reduced the plasma galactose concentrations at 30 and 60 min ($P<0·01$). The plasma glucose response was increased by the addition of glucose to the test meal in the guinea-pigs and mice but not in the rat. The results reported here confirm those of Newstead (1979) and suggest that the mouse also metabolizes galactose differently to man, whereas the guinea-pig might be considered to be a suitable model for the study of galactose metabolism.

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Rat intestinal cytochrome oxidase activity as an indicator of the supply and availability of dietary copper. By J. PRICE and J. K. CHESTERS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The biological availability of different forms of copper has frequently been assessed from the efficiency with which they are utilized by Cu-depleted rats for caeruloplasmin or haemoglobin synthesis or to produce an increase in plasma Cu concentration. However, the sensitivity of such indices to Cu repletion is relatively poor. Histological evidence of an extremely rapid recovery of cytochrome *c* oxidase (*EC* 1.9.3.1) activity in the mucosa from the small intestine of Cu-depleted rats (Dallman & Loskutoff, 1967) prompted an investigation of the sensitivity of this Cu-containing enzyme to Cu repletion.

Twenty male Hooded Lister rats were individually caged 6 d after weaning and offered a low-Cu semi-synthetic diet containing 0·5 mg Cu/kg for 28 d. This depleted the cytochrome *c* oxidase activity in the mucosa of the small intestine to approximately 30% of normal. The rats were then allocated at random to two groups of ten and given, on 3 consecutive days, either 0 or 10 μg Cu as $CuSO_4$ dispersed in 1 g sucrose and added to 9 g of the low-Cu diet. Twenty-four hours after the final Cu dose the rats were killed and the cytochrome *c* oxidase activity assayed by the method of Mills & Dalgarno (1970) in homogenates of the mucosa from a 10 mm section of the proximal duodenum (D), mid-jejunum (J) and terminal ileum (I). The activity of NADH cytochrome *c* reductase (*EC* 1.6.9.3), a second mitochondrial enzyme, was also measured in the same samples. Since the latter enzyme is not Cu-dependent, the ratio of oxidase:reductase was used as the basis for expression of changes in cytochrome *c* oxidase activity.

The mean enzyme activities (with SE) in the unsupplemented and Cu-supplemented groups respectively were 1·38 (0·12) and 3·10 (0·28) (D), 2·94 (0·27) and 4·04 (0·33) (J) and 2·52 (0·18) and 3·77 (0·34) (I). Thus the duodenal cytochrome *c* oxidase showed the greatest response to supplementary Cu.

The relationship between repletion dose of Cu and the response in cytochrome *c* oxidase activity was therefore examined in the duodenal mucosa. Four groups of eight male rats were depleted of Cu as before, then the groups were given 0, 2·5, 5 or 10 μg Cu/rat as $CuSO_4$ on each of 3 consecutive days and killed for enzyme assay 24 h after the final dose. The cytochrome *c* oxidase activity (A) increased linearly with Cu dose (μg Cu/d) as indicated in the following relationship ($P<0·005$):

$$A = 0·124 \ (\text{SE } 0·034) \ Cu + 0·02 \qquad \text{RSD } 0·522$$

Using this technique with groups of eight rats the availability of Cu may be assessed relative to that of $CuSO_4$ in samples providing as little as 15 μg of the element for each rat.

We are grateful to Dr N. T. Davies for advice during the early stages of this work.

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Bioavailability of copper from various fractions of ruminant digesta. By J.
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AB2 9SB*

It is evident that the effect of dietary molybdenum in reducing the utilization of
copper by ruminants depends on modification of dietary components by rumen
microbial activity (Bremner & Davies, 1980). As part of an investigation of the
Mo/Cu antagonism, we have examined the effects of dietary Mo on the bioavail-
ability of Cu from fractions of ruminal and intestinal contents of sheep.

Sieved rumen contents and digesta samples from the proximal duodenum and
terminal ileum were obtained from six sheep given initially dried grass (1·6 mg
Mo, 6·5 mg Cu, 2·9 g S/kg) and then dried grass supplemented with 10 mg Mo/kg
as ammonium molybdate. These samples were separated by anaerobic centri-
fugation into the following fractions: large particles including protozoa (1000 g
pellet), large micro-organisms (2200 g pellet), bacteria (30 000 g pellet) and the
supernatant material.

Groups of eight rats were used to assess differences in bioavailability of Cu as
determined from the response of duodenal cytochrome c oxidase activity (cytox)
when the rats were given 10 μg Cu/d derived from $CuSO_4$ or from digesta fractions
(Price & Chesters, 1984). Results expressed as ($\triangle$ cytox from digesta Cu $\div$ $\triangle$ cytox
from $CuSO_4$) $\times$ 100 are presented in the Table.

Fraction	Rumen		Duodenum		Ileum	
	− Mo	+ Mo	− Mo	+ Mo	− Mo	+ Mo
1000 g pellet	7	−2	10	0	24	−5
2200 g pellet	14	−8	74	−11	20	6
30 000 g pellet	11	−7	102	17	34	10
30 000 g supernatant	40	3	107	50	43	14

The results indicate that the availability of Cu from ruminant digesta was
generally substantially less than that from $CuSO_4$ and that it was further reduced
in all fractions when Mo was added to the diet. Negative availabilities apparently
arose from fractions with unsaturated binding capacity for Cu reducing the
availability of either the low quantities of Cu present in the Cu-deficient diet or
preventing the reabsorption of endogenous Cu.

Previous studies have suggested that reduction in availability of Cu is associated
with the formation of thiomolybdates (Dick *et al.* 1975). Although these anions are
water soluble, they must be largely associated with the particulate matter in
ruminant digesta if they account for the loss of availability of Cu seen in the
present experiments.

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Dietary intake of glucosinolates, mustard oil glycosides. By KAREN SONES and G. ROGER FENWICK, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA* (Introduced by D. A. T. SOUTHGATE)

Glucosinolates are the precursors of the pungent principles of mustard, radish and horseradish. They occur mainly in the Cruciferae, including cultivated vegetables and oilseeds of the genus *Brassica*. Much is known about the (mainly) deleterious properties of these compounds and their hydrolysis products from animal feeding experiments using rapeseed or crambe-seed meals (Fenwick *et al.* 1983). There is, however, very little information available on the physiological effects and metabolism of such compounds in man and only a single report measuring the daily dietary intake (Mullin & Sahasrabudhe, 1978).

Because of the dietary importance of brassica vegetables, especially cabbage, Brussels sprouts, cauliflower and swede/turnip, in the UK the glucosinolate contents of 160 samples of these, obtained from various parts of the country, have been determined for both the fresh and cooked vegetable. These results, together with those available from the Ministry of Agriculture, Fisheries and Food (1982), has allowed the calculation of dietary intakes of both total and individual glucosinolates.

Total glucosinolate content (mg/kg fresh weight) and average dietary intake (ADI; mg/person per d)

| | Glucosinolate content | | | | | |
| | Fresh | | | Cooked | | |
	Mean	Range	ADI	Mean	Range	ADI
Brussels sprout	2260	1460–3940	17·2	1240	600–2540	9·4
Cabbage	1090	360–2750	19·4	790	320–1650	14·0
Cauliflower	620	140–2080	6·4	420	90–1110	4·4
Swede/turnips	560	390–1660	3·1	290	210–940	1·6
Total			46·1			29·4

The daily dietary intake (46·1 mg) is much higher than previously reported from Canada (7·9 mg) (Mullin & Sahasrabudhe, 1978). Average intake varies between 57·1 mg (Southwest England) and 27·1 mg (Scotland) and is almost double in the winter months (58·8 *v.* 32·8 mg). Although brassicas are generally processed before consumption the intake values based on analysis of cooked material do not take account of glucosinolates leached into cooking water which are still available (via gravy or soups).

Average dietary intakes of glucosinolates giving rise to goitrogenic products (Elfving, 1980; Fenwick *et al.* 1983) total 21·4 mg/person per d. It is considered that the dietary intake of individuals may be much higher than the values quoted here, but the nature and extent of any associated risk cannot yet be assessed.

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High-fibre, low-sodium, low-fat dietary treatment in diabetic subjects with intermittent claudication. By P. J. PACY, M. P. TAYLOR, A. J. KUBICKI and P. M. DODSON, *Department of Diabetes, Endocrinology and Lipid Metabolism, Clinical Investigation Unit, Dudley Road Hospital, Birmingham*

Diabetes mellitus is associated with premature atherosclerosis. Treatment of intermittent claudication tends to be poor. Recently there have been reports on the beneficial effect of dietary therapy in this condition.

In a 3 month non-controlled trial we have examined the effect of a high-fibre (40 g/d), low-sodium (40–50 mmol/d) and low-fat (15% of total energy) diet in seventeen diabetic subjects with intermittent claudication (mean (with SD): age 59·4 (5·9) years, ideal body-weight 125·5 (9·1) %, period of diagnosis 12·2 (11·6) years; twelve male, five female, five smokers, six treated with insulin).

No improvement of Doppler ankle: arm systolic blood pressure values (mean (with SD)) (dorsalis pedis: right 0·7 (0·4) *v.* 0·7 (0·4), left 0·7 (0·4) *v.* 0·7 (0·4); posterior tibial: right 0·8 (0·3) *v.* 0·8 (0·3), left 0·8 (0·3) *v.* 0·7 (0·4)) or duration on the treadmill (302·1 (158·6) *v.* 295·4 (173·4) s) was documented. Maximal heart beats/min were similar (128·3 (28·7) *v.* 126·0 (26·3)). Thirteen patients (76·5%) felt subjectively improved.

Significant changes in mean (with SD) systolic (162·4 (24·6) *v.* 147·5 (21·0) mm Hg, $P<0.01$) and diastolic (83·6 (16·9) *v.* 77·4 (15·1) mm Hg, $P<0.05$) blood pressures, daily urinary Na (188·1 (22·0) *v.* 117·4 (15·3) mmol, $P<0.001$), urinary Na:K value (3·2 (0·4) *v.* 2·3 (0·4), $P<0.02$), serum triglyceride (1·8 (0·2) *v.* 1·5 (0·1) mmol/l, $P<0.02$), glycosylated haemoglobin (12·5 (0·7) *v.* 10·2 (0·8) %, $P<0.001$) and weight (76·5 (2·2) *v.* 73·3 (2·2) kg, $P<0.001$) occurred.

Ten of the patients were also hyperlipidaemic on initial investigation. After 3 months, similar significant changes were noted including a reduction in serum cholesterol (6·9 (0·7) *v.* 5·9 (0·6) mmol/l, $P<0.05$) and triglyceride (2·1 (0·2) *v.* 1·6 (0·2) mmol/l, $P<0.02$).

Although the exact extent of compliance cannot be assessed, we conclude that a high-fibre, low-Na and low-fat diet may not increase claudication distance although there was symptomatic improvement with reduced cardiovascular risk in diabetic subjects with peripheral vascular disease.

Bile acid binding and the cholesterolaemic effect of dietary proteins. By
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Considerable attention has been given to the hypocholesterolaemia produced by
soya-bean protein in comparison with animal proteins such as casein. This effect is
accompanied by increased steroid excretion and may involve structural properties
of the proteins (West *et al.* 1983). Undigested dietary proteins are known to bind
bile acids and also to decrease bile acid reabsorption from duodenal loops (Sklan
et al. 1979). As a possible mechanism of cholesterolaemia, we have thus studied
bile acid binding and precipitation in vitro by soya-bean protein (Supro 500E,
Ralston Purina Co.) and casein (acid casein, DMV BV, The Netherlands).

Precipitation was measured by adding protein to 50 mM sodium cholate in
NaH_2PO_4/Na_2HPO_4 buffer (100 mM; pH 7·4). These suspensions were incubated
for 30 min and centrifuged (10 000 *g* for 15 min) before measuring levels of bile
acid (Boyd *et al.* 1966) and protein (Lowry *et al.* 1951) in the supernatant. There
was a lower bile acid concentration in the supernatant of incubations containing
soya-bean protein than in those with casein. Supernatant protein concentrations
were also lower for soya-bean. Bile acid binding was measured using equilibrium
dialysis. 500 mg protein and 4 ml buffered cholate solution were confined in
duplicate dialysis tubes and equilibrated with 6 ml buffered cholate. The average
final bile acid concentrations in the dialysate were 25 mM and 24 mM for soya-bean
and casein respectively.

*Cholate and protein concentrations in supernatant of incubations containing
casein or soya-bean protein*

	Initial protein concentration in incubation (g/l)			
	5	10	50	100
Cholate (mM)				
Casein	49	48	41	38
Soya-bean protein	43	40	29	24
Protein (g/l)				
Casein	3	6	27	62
Soya-bean protein	2	2	7	13

Thus, although bile acid binding is similar for casein and soya-bean protein, the
latter produces a greater bile acid concentration in the precipitate because of its
insolubility. In vivo bile acid precipitation by soya-bean protein may be enhanced
by a slow rate of hydrolysis, also due to low solubility (C. J. H. Woodward and
K. K. Carroll, unpublished results). Such effects could increase bile acid excretion
and contribute to hypocholesterolaemia.

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Source of peripheral blood ammonia in ruminants and its relationship to clinical ammonia poisoning. By MARGARET I. CHALMERS and F. WHITE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Clinical ammonia poisoning in ruminants is associated with the feeding of urea in sufficient amounts to raise the rumen pH over 7·2 and to increase the ruminal ammonia concentration in excess of 0·6 gN/l (Chalmers *et al.* 1971). Recently it has been suggested that ammonia poisoning could arise in high-yielding dairy cows eating fresh grass (Symonds *et al.* 1981). First signs of ammonia poisoning are seen when the arterial blood ammonia concentration exceeds 8 mg N/l.

The relationship between the concentrations of ammonia in the aorta (A) and portal vein (P) blood was studied in maiden ewes given (I) urea directly into the rumen or (II) an infusion of ammonium salts into the anterior mesenteric vein. Under (I) all of the results could be described by the single relationship A = 0·408 P (RSD 0·71), but under (II) the relationship changed significantly when portal blood concentrations exceeded 9 mg ammonia N/l. The relationships are not statistically combinable between I and II.

Source of ammonia	Number of:			Concentration of ammonia in portal vein blood (mg N/l)		Relationship between aorta (A) and portal vein (P) blood ammonia concentrations
	Sheep	Experiments	Values	Mean	Range	
I. Urea of rumen	7	10	24	6·44	2·5– 9·0	A = 0·45 P, RSD 0·56
			65	13·02	9·0–17·5	A = 0·39 P, RSD 0·7
II. Infusion of ammonium salts into mesenteric vein	4	12	66	5·74	2·5– 9·0	A = 2·24, SD 0·28*
			103	15·12	9·0–26·0	A = 0·165 P + 0·56, RSD 0·56

*No significant relationship to P.

Further measurements were made on sheep eating natural feeds, dried grass, barley cubes, fish meal with cereals and hay, or fresh grass to appetite. In these animals the ruminal ammonia concentration did not exceed 0·3 g N/l, rumen pH was less than 7, and the maximum ammonia concentration in portal vein blood was 11 mg N/l equivalent to 1·4 mg N/l in arterial blood (eqn (II)). The concentration of ammonia in aorta ranged from 2·1 to 2·8 mg N/l. In twenty milking cows eating fresh grass, the concentration of ammonia in jugular vein blood averaged 2·8 mg N/l in June 1982 and 2·9 in August 1982.

Clinical ammonia poisoning is not a problem in normal agricultural practice but care may be needed when urea is incorporated into feeds. Any existing disease which interfered with the excretion of N could override these conclusions.

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Milk production in cows infused abomasally with casein, glucose or aspartic and glutamic acids early in lactation. By J. D. OLDHAM and J. A. BINES, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT* and J. C. MACRAE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Casein infused into the abomasum of cows in negative energy balance early in lactation can increase milk production and exaggerate the negative energy balance (Ørskov *et al.* 1977). Abomasal casein infusion has also been found to increase whole-body flux rates of palmitate and acetate (Konig *et al.* 1981). The constituents in casein which induce these changes have not been identified but one hypothesis is that constituent amino acids may act by priming the tricarboxylic acid cycle to facilitate metabolism of two-carbon units which might allow or enhance mobilization of body fat.

To test this hypothesis, four mature Friesian cows were infused abomasally with (i) saline (9 g sodium chloride/l), (ii) casein (300 g/d), (iii) glucose (425 g/d) or (iv) a mixture of aspartic (90 g/d) and glutamic (310 g/d) acids. The experiment was a 4×4 Latin-square with 9-d periods starting at 9 d post-partum. The cows were offered a low-protein ration (g/kg: 600 concentrates, 400 hay) and were, by calculation, in negative energy balance at the start of the experiment.

	Yield				g/l Milk		
Treatment	Milk (l/d)	Fat (g/d)	Protein (g/d)	Lactose (g/d)	Fat	Protein	Lactose
(i) Saline	24·4	960[b][c]	714[b]	1173	39·1[a][b]	29·4[b]	48·1
(ii) Casein	26·6	1110[a]	812[a]	1250	41·7[a]	31·0[a]	47·8
(iii) Glucose	24·5	884[c]	710[b]	1152	36·2[b]	29·1[b]	47·1
(iv) Aspartic/ glutamic acids	24·9	990[b]	749[a][b]	1190	39·8[a][b]	30·2[a][b]	47·7
SED (6 df)	0·97	42·0	38·0	57·2	1·89	0·65	0·49

[a],[b],[c] Means which do not share a common superscript differ significantly: $P < 0.05$.

Casein infusion significantly ($P < 0.05$) increased yields of milk fat and milk protein and decreased calculated energy balance from −19 MJ metabolizable energy (ME)/d to −30 MJ ME/d compared with the saline (control) infusion. Infusion of the aspartic/glutamic acids mixture had no effect on the yield of milk constituents but calculated ME balance was −24 MJ ME/d, largely because one cow refused 17% of the ME allowance during the infusion of aspartic/glutamic acids.

We conclude that in these circumstances aspartic and glutamic acids in casein were not the causal agents of enhanced milk production and energy deficit.

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The influence of gastrointestinal cannulation on the energy metabolism of the pig. By W. H. CLOSE*, R. P. HEAVENS and D. B. STEPHENS, *AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT* and I. E. SAMBROOK, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Animals prepared with gastrointestinal cannulas have been extensively used for studying the processes of digestion along the gastrointestinal tract. It is assumed that the information obtained from these animals is directly applicable to intact animals. Whereas the effects of gastrointestinal surgery on the metabolic rate of sheep have been investigated (MacRae *et al.* 1982; Close *et al.* 1984), no such information is available for the pig. Our current studies on volatile fatty acid metabolism provided the opportunity to determine whether the energy metabolism of the pig might be altered by cannulation of the caecum.

Two pairs of Large White pigs, initial body-weight 25 kg, were kept at an environmental temperature of 20° (±0·5) and fed on a conventional cereal-based diet, according to the Shinfield scale of feeding (Barber *et al.* 1972). Following suitable habituation to the experimental conditions and protocol, each pair of animals was removed to heat-sink calorimeters for a 7-d period when their heat loss and energy and nitrogen balances were individually determined. Immediately afterwards, one pair of animals was prepared with a caecal cannula, while the remaining pair (controls) was laparotomized. Following a 14-d period of recovery, the animals' heat loss and energy and N balances were measured for a further 7-d period.

The partition of the metabolizable energy (ME) intake into components of heat loss (H), energy retention (ER) and protein (P) and fat (F) deposition is presented in the Table.

	Body-weight (kg)	ME	H	ER	P	F
		(kJ/kg body-weight$^{0.75}$ per d)				
Cannulated						
Before	37·9	1368	776	592	216	376
After	47·8	1363	803	560	218	342
Control						
Before	47·8	1336	812	524	207	317
After	62·5	1385	802	583	222	361

When variations in ME intake were accounted for, there was little difference in the values of energy metabolism, suggesting that in the present experiment there was no significant effect of cannulation on the metabolic status of the pigs.

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The energy cost of pregnancy. By W. H. Close[*], *AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

The determination of the energy requirements during pregnancy requires knowledge of the requirements for maintenance (ME_M) and for net maternal and reproductive tissue deposition. There is little information to show whether there are differences in the energetic efficiency of tissue deposition during pregnancy and estimates of these were made in studies relating to the energy metabolism of the pregnant sow.

The energy retention (ER) in pregnant gilts was partitioned into reproductive (gravid uterus and mammary tissue; ER_R) and maternal (ER_M) components at metabolizable energy (ME) intakes of 20 and 30 MJ/d at three stages of gestation when kept at an environmental temperature of 20°. Since non-pregnant litter sisters were also investigated, the rates of maternal tissue accretion in both pregnant and non-pregnant animals were compared.

The net energetic efficiency during pregnancy (k_{preg}) was calculated by three separate methods:

1. k_{preg} was calculated from data relating to non-pregnant animals, assuming that the maternal tissue of pregnant animals have similar requirements for both maintenance and maternal tissue deposition as non-pregnant animals. Net efficiency values of 0·52 and 0·61 were calculated depending on the data included in the analysis.

2. Multiple regression analyses were used to partition ME intake into components of ME_M, ER_M and ER_R (kJ/kg body-weight$^{0.75}$ per d). The equation was:

$$ME = 424 + 1·15 + 1·39\ ER_R$$

The reciprocals of the coefficients, that is 0·87 and 0·72, represent the respective energetic efficiencies for maternal and reproductive tissue deposition.

3. This method makes use of the difference in heat production between pregnant and non-pregnant animals, that is the heat increment of gestation. At the different stages of gestation and levels of ME intake, values of k_{preg} of between 0·38 and 0·71 were calculated.

Depending upon the method of calculation, net efficiency values between 0·38 and 0·72 were determined for the reproductive tissue, with the average being 0·63. This compares with the best estimate of 0·69 determined during growth and fattening (Agricultural Research Council, 1981). Theoretical estimates based on the rates of protein and fat deposition at the different stages of gestation indicate a k_{preg} value of 0·60, in agreement with the average of 0·63 from the present study.

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Defective sympathetic regulation of brown adipose tissue of Zucker obese rats. By S. HOLT, D. MARCHINGTON and D. A. YORK, *Department of Nutrition, Southampton University, Southampton SO9 5NH*

The energy imbalance of Zucker fa/fa rats has been associated with the loss of brown adipose tissue (BAT) mitochondrial thermogenic response to dietary stimuli. The response to environmental temperature remains intact (Holt *et al.* 1983). Adrenalectomy normalizes energetic efficiency of the fa/fa rat (Marchington *et al.* 1983) and restores the BAT mitochondrial function and its response (increase in GDP binding) to supplementary dietary sucrose (Holt & York, 1982; Holt *et al.* 1983). The present experiments have investigated the role of the sympathetic nervous system in the loss of BAT mitochondrial function.

Lean (Fa/?) and obese (fa/fa) rats (4–5 weeks old) were housed at 22° in a 14–10 h light–dark cycle. BAT mitochondria (BATM)-[^{3}H]GDP binding was assayed as described previously (Holt *et al.* 1983). Obese rats showed a normal increase in GDP binding 40 min after noradrenaline (500 μg/kg subcutaneously). Propranolol (20 mg/kg; 1, 10 and 18 h before killing) reduced BATM-GDP binding of lean rats to that of obese littermates and inhibited the increase in GDP binding normally associated with adrenalectomy (ADX) of fa/fa rats (Table).

[^{3}H]GDP binding to BATM (pmol/mg protein)

	Lean		Lean-ADX		Obese		Obese-ADX	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
−Propranolol	313·4	11·8	314·2	2·2	158·9	7·7	258·8	4·9
+Propranolol	190·9	1·9	177·6	4·3	143·2	6·1	172·1	15·8

In addition both the concentration and turnover of noradrenaline in BAT of obese rats is reduced. The mean (with SE) noradrenaline content of BAT was reduced in obese rats (0·14 (0·01) *v.* 0·28 (0·02) μg/tissue). No turnover of noradrenaline could be measured in BAT of obese rats ($t_{\frac{1}{2}}$ 4·2 h in lean rats).

The results suggest that the defective BATM-GDP binding observed in obese rats results from an impairment in the sympathetic stimulation of that tissue.

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The effect of age and gene-dosage on brown adipose tissue of Zucker obese fa/fa rats. By D. A. York and S. Holt, *Department of Nutrition, Southampton University, Southampton SO9 5NH* and N. J. Rothwell and M. J. Stock, *Department of Physiology, St. George's Hospital Medical School, Tooting, London SW17 0RE*

Excess fat deposition has been reported in 10-d-old preobese Zucker fa/fa rats (Boulange *et al.* 1979), before any detectable rise in serum insulin or food intake. The excess energy deposition of weaned fa/fa rats has been related to the increase in energetic efficiency resulting from a lack of diet-related brown adipose tissue (BAT) thermogenesis (Holt *et al.* 1983; Marchington *et al.* 1983). Experiments were performed to investigate (i) if this defect in the regulation of BAT thermogenesis is also responsible for the initial energy imbalance in suckling fa/fa pups, and (ii) to see if BAT function was related to genotype.

BAT mitochondria (BATM) were prepared and the specific binding of $[^3H]$GDP assayed by previously described methods (Holt *et al.* 1983). In addition, the thermic response ($\dot{V}O_2$) to a 50 kJ Complan® meal was measured by indirect calorimetry (Rothwell *et al.* 1983).

Both BATM-GDP binding and the metabolic response ($\dot{V}O_2$) to a Complan meal showed a fa gene dependency being intermediate in lean Fa/fa heterozygotes between homozygous (Fa/Fa) lean and homozygous (fa/fa) obese groups (see Table).

BAT mitochondrial function and metabolic response to a Complan meal

	Fa/Fa		Fa/fa		fa/fa	
	Mean	SE	Mean	SE	Mean	SE
BATM-GDP binding (pmol/mg)	355	9	236	10	128	12
Increase in $\dot{V}O_2$ (%) after Complan	21·0	1·6	12·7	1·4	7·2	1·0

BATM-GDP binding was reduced in suckling preobese fa/fa rats at 14 d of age and remained depressed throughout growth.

The early appearance and fa gene dependency of defective BAT mitochondrial function suggests that the impaired diet-related BAT mitochondrial thermogenesis may be closely related to the expression of the defective fa gene.

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Thermic effects of methyl cellulose. By N. J. ROTHWELL, M. J. STOCK and
   B. P. WARWICK, *Department of Physiology, St. George's Hospital Medical
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The acute rise in metabolic rate after a meal (thermic effect, TE) is partly due to
sympathetically-mediated thermogenesis and is dependent on nutrient composition
and the thermogenic status of the animal (Rothwell *et al.* 1982; Rothwell & Stock,
1983). Thermic responses to food have always been thought to follow nutrient
absorption, but in this study we have investigated the acute TE of a non-nutrient
load (methyl cellulose).

Thermic responses to carbohydrate (CHO; 40 kJ cornflour; 4 ml slurry in water)
and methyl cellulose (MC; 70 g/l water, 4 ml) were measured in male Sprague-
Dawley rats (200–300 g). Resting oxygen consumption ($\dot{V}O_2$) was measured for 2 h
before and after intragastric feeding in closed-circuit respirometers maintained at
29°. Both meals produced significant ($P<0.01$) increases in post-prandial $\dot{V}O_2$
(mean (with SE)) (CHO: 21.8 (3.1) %; MC: 18.0 (3.0) %) and these responses
were significantly ($P<0.05$) reduced (CHO: 9.0 (1.5); MC: 6.9 (1.9), post-prandial
% increase in $\dot{V}O_2$) by β-adrenergic blockade (propranolol: 20 mg/kg
subcutaneously, 2 h before, and at the time of intubation).

Recent evidence (Rothwell *et al.* 1983) suggests that insulin release is
responsible for the increase in sympathetic activity following CHO feeding, and
inhibition of pancreatic insulin release (diazoxide, 45 mg/kg subcutaneously,
30 min before a meal) was found to inhibit the thermic responses to CHO and MC
to the same extent as propranolol (post-prandial % increase VO_2: CHO before
18.3 (4.2), after diazoxide 2.5 (2.7); MC before 22.2 (3.6), after 6.0 (2.5)). The
TE of an isoenergetic (but hypovolumetric, 1.2 ml) fat meal (maize oil) was
unaffected by diazoxide (post-prandial % increase $\dot{V}O_2$: 23.3 (3.2) before, 20.6
(1.9) after diazoxide).

Since MC is non-metabolizable, part of the thermic response to intragastric
feeding presumably results from gastric distension and involves pancreatic insulin
release and sympathetic activation of thermogenesis. This insulin-dependent phase
of the TE is absent when small volumes of fat are given, although the total TE may
be greater. It is possible that a vagal reflex is responsible for the release of insulin
following ingestion of food and/or stomach distension.

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Effects of selective β-adrenoreceptor agonists on energy balance and body composition in the rat.

By N. J. Rothwell, M. J. Stock and P. D. O'B. Winter, *Department of Physiology, St. George's Medical School, Tooting, London SW17 0RE*

An examination of the acute effects of various adrenergic agents revealed that thermogenesis is most responsive to agonists with mixed β-receptor activity (Rothwell *et al.* 1983). In the present study we have investigated the chronic effects of selective adrenergic agents on energy balance and body composition in young male rats maintained on a standard pelleted diet.

In the first experiment, administration (1 mg/kg per d) of either noradrenaline bitartrate (NA; mixed α, β; Sigma), isoprenaline sulphate (ISO; mixed β; Sigma), prenalterol hydrochloride (PREN; β_1; Hassle), clenbuterol hydrochloride (CLEN; β_2; Boehringer Ingelheim) or vehicle alone (control; CON) for 18 d produced slight increases in metabolizable energy intake, body-weight gain and energy expenditure in the ISO-, PREN- and CLEN-treated groups, when compared with the controls, but a slight decrease in these indices for the NA-treated group. Both NA and ISO treatment significantly reduced net energetic efficiency (NE) and, although PREN and CLEN produced a slight decrease, this was not significantly different from control values (mean (SE) NE %: CON 40 (1), PREN 35 (1), CLEN 35 (1), NA 32 (3), ISO 31 (2); *n* 6). Body composition was unaffected by PREN and NA treatment, although body water (g water: CON 183 (3), CLEN 203 (5), ISO 194 (5)) and body protein (g protein: CON 48 (1), CLEN 55 (1), ISO 51 (1)) were increased and body fat (g fat: CON 20 (1); CLEN 18 (1); ISO 14 (1)) decreased by CLEN and ISO treatment.

In the second experiment, the chronic administration of clenbuterol and fenoterol (FEN; β_2; 2 mg/kg per d) for 18 d produced significant increases in body-weight gain (CLEN 27%, FEN 15%; *n* 8, $P < 0.01$) and metabolizable energy intake, although this was only significant for the clenbuterol group (kJ/d: CON 864 (38), CLEN 976 (21), $P < 0.05$). Both drugs increased body energy gain (CLEN 10%, FEN 5%), although clenbuterol also significantly increased energy expenditure (CLEN 16%; $P < 0.05$) and decreased net energetic efficiency (CLEN −26%; $P < 0.05$). Body water content (% above control: CLEN 11%, FEN 10%) and body protein (% above control: CLEN 24%, FEN 10%) were both significantly affected by drug treatment.

These effects of β_2-agonists on body protein and fat contents, which were not seen in response to β_1-agonists, could be of value in the treatment of obesity in man and in the production of leaner livestock.

Rothwell, N. J., Stock, M. J. & Winter, P. D. O'B. (1983). *Journal of Physiology* **340**, 62P.

Effect of physiological state on response of female rats to cafeteria-feeding. By H. GILLIAN BARR and K. J. McCRACKEN, *Department of Agricultural and Food Chemistry, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*

Increased energy intake and the anatomical and compositional changes accompanying pregnancy and lactation are well documented (Cripps & Williams, 1975), but less attention has been devoted to post-lactation changes. This experiment was designed to investigate the effect of physiological state on the response of rats to pelleted diets or cafeteria feeding (Scalafani & Springer, 1976).

Sixty Sprague-Dawley rats housed singly at 24°, of initial weight 310 g, were blocked for weight and twenty allocated to each of three physiological conditions: unmated (P1), lactating (P2), non-lactating (P3). All P2 and P3 rats became pregnant. At parturition, pups of P3 dams were culled and litters of P2 dams adjusted to eight. P2 rats lactated for 21 d, during which time all rats were fed on a low-fat diet (LF) *ad lib*. Prior to weaning, rats were weighed, blocked within their initial group and allocated to dietary treatment C (control LF), CAF (cafeteria diet) or slaughtered (S) (5, 10, 5 animals respectively). Metabolizable energy (ME) intake was measured for 42 d after which rats were killed and carcass composition and energy retention (ER) determined as described by McCracken & Barr (1982).

| | Unmated | | Lactating | | Non-lactating | | SED | Statistical significance |
	C	CAF	C	CAF	C	CAF	(n 5)	($P<$)
Weight gain (g/d)	1·3	3·4	0·8	3·2	1·2	3·2	0·49	0·001
ME intake (MJ/d per kg body-weight$^{0.75}$)	0·65	0·79	0·63	0·88	0·65	0·78	0·037	0·001
ER (MJ/d per kg body-weight$^{0.75}$)	0·02	0·17	0·09	0·24	0·04	0·20	0·035	0·001

CAF increased intake, weight gain and ER in all physiological states, P2CAF rats eating significantly more than other groups. There was no significant difference in the intakes of control groups but energy retention of P2C animals tended to be higher and the energy content of the gain was significantly higher.

These results suggest that lactation may increase the efficiency of energy utilization above that prior to mating and significantly affect the response of rats to organoleptic stimuli, thus leading to increased fat deposition during the post-weaning period.

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Unimpaired capacity for thermogenesis in genetically obese (ob/ob) and hypothalamically obese (MSG) mice. By A. G. DULLOO and D. S. MILLER, *Department of Nutrition, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH*

The response to administration of high doses of noradrenaline has frequently been used for quantitative determination of the capacity for non-shivering thermogenesis (Himms-Hagen, 1976). Such a capacity has often been reported to be lower in the genetically obese ob/ob mice, and this has led to the suggestion that the elevated energetic efficiency involved in the development of obesity in these mutants is largely attributed to a reduction in thermoregulatory non-shivering thermogenesis (Trayhurn & James, 1978).

In the present study, the thermogenic response to noradrenaline administration was investigated at 25° in two different models of obese mice (the genetic ob/ob obesity and monosodium glutamate (MSG)-induced hypothalamic obesity) and in their respective lean littermates. Each group consisted of five male mice, aged 3–4 months. Resting oxygen consumption was monitored individually for 1 h before and 2 h after subcutaneous injection of noradrenaline (Levophed; Winthrop, England).

Administration of a relatively low dose of noradrenaline (100 μg/kg body-weight) was without effect in the lean animals but caused about 30% increase in the peak oxygen consumption in both obese types ($P<0.001$ v. lean). In contrast, the elevation in peak metabolic rate after injection of a high dose of noradrenaline (600 μg/kg body-weight) was of the same magnitude in both lean and obese animals within each model: in the hypothalamic model the mean (with SE) metabolic rate was increased by 80 (7) % in the obese compared with 71 (9) % in the lean controls, while in the genetic ob/ob model the metabolic rate was elevated by 55 (3) % and 49 (2) % in the obese and lean animals respectively. A similar unimpaired response to the thermogenic effects of noradrenaline in the ob/ob mouse has also been reported at 29° (Macdonald & Stock, 1979) and at 31° (Arch, 1983).

The results presented here indicate that at 25° the over-all whole body capacity for thermogenesis is unimpaired in the genetic ob/ob and hypothalamic MSG obese models and suggest that the metabolic defect in the obese mice is due to defective neural and/or endocrine mechanisms rather than a defect in the thermogenic tissues: their problem, simplistically, is a failure to release noradrenaline rather than a lack of response to noradrenaline.

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Differential changes in free and total 3,5,3′-triiodothyronine in cold-exposed rats. By J. G. BROWN, J. VAN BUEREN and D. J. MILLWARD, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

Although thyroid hormones have long been known to play important roles in energy and protein metabolism, there are still major gaps in our understanding of their mechanism of action. One example is their role in the thermogenic activation in response to cold when although thyroid hormones might be expected to participate in the increased heat production, concentrations of the hormones are not always observed to increase in response to the cold (Rothwell & Stock, 1978). We believe that the equivocal nature of thyroid hormones' role results from inappropriate measurements of thyroid status.

Most studies in which thyroid status is an important consideration, involve measurements of total 3,5,3′-triiodothyronine (T_3) (and often thyroxine) to define thyroid status. However, it is now believed that it is the free fraction of thyroid hormones which is physiologically active and this is seldom measured. Furthermore, disproportionate changes in the free and bound fractions of the hormones can occur. For example, in response to protein deficiency in the rat total T_3 is increased but free T_3 falls in line with the reduced metabolic rate (Cox *et al.* 1981). We report here a further example which has arisen during our studies of the role of thyroid hormones in the regulation of protein metabolism.

We have examined the changes in muscle protein metabolism and thyroid status during cold adaptation in adult female rats (255–265 g) kept at 4° (CA) for 7 and 14 d in comparison with 24° (WA). Cold exposure caused a loss of body-weight and muscle mass during the first 7 d which was regained during days 7–14. The increased heat production of the cold adaptation involved a >90% increase in food intake within 2 d of cold exposure, sustained throughout the experiment. The rates of muscle protein synthesis and degradation in the CA rats were significantly increased, with degradation in excess of synthesis at 7 d but falling subsequently to a level which, although still elevated, was just below the elevated synthesis rate. The increases in protein synthesis and degradation were accompanied by changes in the concentration of muscle RNA and in lysosomal cathepsin D.

Although these changes in protein metabolism are consistent with a hyper-thyroid state (Brown & Millward, 1983), the concentration of circulating total T_3 showed no change during this experiment (WA 0·89 (SE 0·14) ng/ml *v.* CA 0·88 (SE 0·13) ng/ml). However, free T_3 concentrations were increased by 60–80% (WA 5·5 (SE 1·2) pg/ml *v.* CA 9·6 (SE 1·4) pg/ml). This is another example of disproportionate changes in free and total T_3 concentrations demonstrating the inadequacy and often misleading nature of measurements of total T_3 as an index of thyroid status.

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The effect of corticosterone treatment on heat production in the growing rat. By PENNY COYER, M. COX, J. P. W. RIVERS and D. J. MILLWARD, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

Despite the evidence that corticosteroids apparently inhibit thermogenesis (Galpin *et al.* 1983), catabolic doses of corticosterone in young growing rats suppress growth without major changes in body composition and with food intake maintained or even elevated (Odedra *et al.* 1983). This suggests that increased heat production occurs. We have therefore measured heat production in rats treated with moderate and high doses of corticosterone.

Weight-matched groups of male Lister hooded rats (108±1 g initial weight), individually caged on wire grids and given a diet (200 g casein/kg) *ad lib.* were injected subcutaneously with vehicle containing (a) 0, (b) 50 or (c) 100 mg corticosterone/kg body-weight (BW) daily for 5 d. Metabolizable energy (ME) intake was calculated as the difference between energy content of the total food given and that in the dried spillage, faeces and urine. Energy deposition (D) was determined as the difference between energy content of an initial group and the groups killed after 5 d. Heat production (H) was calculated as the difference between ME intake and D. H was also estimated at 6 h after feeding from daily measurements of oxygen consumption in similarly treated groups.

	Control (n 6)		50 mg corticosterone/ kg BW per d (n 6)		100 mg corticosterone/ kg BW per d (n 6)	
	Mean	SE	Mean	SE	Mean	SE
ME intake (kJ/BW$^{0.75}$ per d)	1032	10·9	746·6	17·7	668·8	54·5
Heat production (kJ/BW$^{0.75}$ per d)	648·8	19·8	533·2	10·8	735·8	30·1
Mean BW (g)	124·6	2·5	104·8	3·0	99·9	2·3

Corticosterone treatment resulted in a depressed ME intake which was due to urinary energy losses resulting from the massive glycosuria. D was reduced by 40% and H was depressed by 18% at dose (b) while energy deposition was completely abolished with no fall in H at dose (c). Oxygen consumption was unchanged at dose (b) and elevated at dose (c).

According to classical concepts of partition of H between components related to BW (maintenance) and D (growth costs), growth suppression should induce a fall in H due to reduced growth costs. In the dose (b) group the magnitude of the fall in H was in the expected range for the reduced growth costs, whereas in the dose (c) group, the total growth suppression was not accompanied by any fall in H. This interpretation of our findings indicates that in the rat, corticosterone at doses (b) and (c) does not specifically suppress H. Indeed at the higher dose H was increased.

Galpin, K. S., Henderson, R. G., James, W. P. T. & Trayhurn, P. (1983). *Biochemical Journal* 214, 265–268.
Odedra, B., Bates, P. C. & Millward, D. J. (1983). *Biochemical Journal* 214, 617–627.

Zinc homeostasis in the severely Zn-deficient rat. By R. Giugliano and D. J. Millward, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

Zinc is a constituent of many enzymes and is therefore a key essential nutrient. Although it is generally believed that there are no substantial Zn stores in the body, growth does not stop immediately when rats are given a Zn-deficient diet. We have examined therefore the growth and Zn content of several tissues in severely Zn-deficient rats in order to better understand Zn homeostasis. A very low-Zn diet was prepared from egg albumin (0.4 mg Zn/kg diet) and this was compared with a control diet (55 mg Zn/kg diet).

The low-Zn diet induced a slowing of growth and the characteristic cyclic losses and gains in body-weight subsequent to changes in food intake. Ratios of muscle mass:body-weight were reduced in the deficient rats compared with pair-fed rats, and rats given two different restricted intakes of the control diet. Thus the Zn deficiency per se induced a specific muscle growth depression. Tissue Zn concentrations during a 24 d period on the deficient diet fell in most tissues, particularly in bone (to 24% of the Zn-supplemented values), but muscle and thymus showed no fall in Zn concentrations. In fact the increase in muscle mass resulted in an increase of 64% in the total muscle Zn pool, of which only one-third could have been derived from the diet and the rest primarily from bone. It would appear therefore that Zn can be partially provided for muscle growth by mobilization from bone.

The constancy of the muscle mass:body-weight value during the cyclic changes in body-weight indicated that cyclic changes in muscle mass occurred. Over the entire 24 d period the total weight loss occurring in the catabolic phases (i.e. the sum of all the catabolic episodes) was such that had it not been balanced by the anabolic phases, 30% of body-weight would have been lost. Although Zn losses can be great in catabolic states, in these experiments Zn losses in the catabolic phases must have been very small since Zn was conserved in the whole body during the redistribution from bone to muscle. While plasma Zn was higher in the catabolic than in the anabolic phase of the cycle indicating some loss from tissues, the increase was very small, the concentration never achieving 50% of control levels. This suggests that in the Zn-deficient rat, mechanisms exist to selectively retain Zn in tissues during catabolic periods. A consequence of this would be an increase in Zn concentration during the catabolic phase of the cycle. Our measurements did indicate an increase (4%) of the expected order in the Zn concentration in muscle although this was not statistically significant. Nevertheless, the results as a whole suggest that mechanisms exist to conserve Zn during Zn deficiency, while making it available for the growth of muscle by mobilizing it from tissues such as bone.

Muscle protein turnover in the severely zinc-deficient rat. By R. GIUGLIANO and D. J. MILLWARD, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, 4 St. Pancras Way, London NW1 2PE*

The cyclic changes in food intake and tissue growth which are a feature of zinc deficiency in the rat, allow the study of not only the effects of Zn-deficiency on protein metabolism but also the mechanisms by which food intake regulates tissue growth. We report here measurements of muscle protein synthesis (by the large dose phenylalanine method), degradation (as the difference between synthesis and net change in protein), 3-methyl histidine (3MH) excretion, and changes in plasma insulin and corticosterone secretion in well-fed (BL), Zn-deficient (ZD) and pair-fed control (CR) rats.

After 10 d on the diet, protein synthesis in muscle fell markedly in both ZD and CR groups from the BL values, but the rate did not differ significantly between ZD and CR groups. However, in ZD rats (n 12) the cycling of body-weight was very marked and there was considerable variability between rats in muscle protein synthesis ($2 \cdot 3-11 \cdot 5$ g protein synthesized/d per g RNA compared with BL values of $16 \cdot 1$ (SE $0 \cdot 4$) g protein synthesized/d per g RNA) and the highest values were observed in animals in the anabolic phase of the cycle. Rates of protein degradation were also variable and were inversely correlated with the growth rate of the rat. Thus changes in both processes contributed to the cyclic changes in muscle mass. However, after 17 d, when cycling was less marked, changes in degradation alone could account for the cycling. Changes in muscle free 3MH and 3MH excretion were inconsistent and could not be interpreted, at least by us.

The cyclic changes in muscle protein synthesis were inversely related to plasma Zn concentrations indicating that changes in Zn status were not directly responsible for the changes in muscle protein metabolism. Although plasma insulin levels in the ZD rats at 10 d were lower than in CR rats ($P<0 \cdot 05$) no variability was observed. In contrast, urinary corticosterone excretion rates varied markedly and inversely with the cyclic changes in body-weight. Furthermore, adrenalectomized ZD rats exhibited higher mortality than in intact ZD rats and exhibited a reduced cycling of food intake and daily weight gain between 11 and 18 d on the diet.

These results indicate that Zn deficiency limits the maximum translational phase of protein synthesis, but does not completely prevent muscle protein synthesis and degradation responding to changes in food intake. These responses appear to be mediated by changes in corticosterone, which in turn may influence protein synthesis by antagonizing the anabolic stimulus of insulin (Bates *et al.* 1984) and directly stimulating protein degradation (Odedra *et al.* 1983).

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Odedra, B., Bates, P. C. & Millward, D. J. (1983). *Biochemical Journal* **214**, 617–627.

Handling of vitamin C by elderly patients. By H. L. LIM and R. J. NEALE, *Department of Applied Biochemistry and Food Science, School of Agriculture, Sutton Bonington, Leics LE12 5RD* and J. R. KEMM, *Department of Social Medicine, University of Birmingham*

Many authors have remarked that plasma levels of vitamin C in elderly patients were commonly low (Vir & Love, 1981; Kemm & Allcock, 1984). These low values could be due to low dietary intakes or to differences in the handling of vitamin C by the elderly (Hughes, 1980).

We have explored this question using an ascorbic acid tolerance test. Subjects were given an oral dose of 1 g crystalline ascorbic acid. Urine was collected for 2 h prior to dosing and then at hourly intervals for the first 4 h and thereafter at longer intervals. Venous blood samples were taken 0, 2 and 4 h after dosing in all subjects and also at 1, 6 and 22 h after dosing in the young subjects. The subjects avoided vitamin-C-rich foods on the day preceding and the day of the test but their diets were not otherwise restricted. Blood and urine samples were preserved by addition of metaphosphoric acid and frozen until analysis. In order to ensure complete collection in the elderly, only patients who had been catheterized were studied and preservative was added to the collecting bag prior to use. Vitamin C in samples was measured by a modification of the method described by the Department of Health and Social Security (1979).

In ten healthy young subjects (five male, five female, age 21–39 years) plasma vitamin C rose by 10 mg/l (mean; range 5–16) reaching the highest value at 2 or 4 h. In the first 6 h, 12% (mean; range 5–19%) of the dose was excreted in the urine. Five of the young subjects (three male, two female) were tested again after 1 month's supplementation with 1 g vitamin C/d. In the first 6 h 18% (mean; range 14–23%) of the dose was excreted.

In nine elderly subjects (two male, seven female, age 75–98 years) hospitalized for various diseases but not clinically scorbutic or known to malabsorb plasma vitamin C, levels rose by 6 mg/l (mean; range 1–10). None of the three subjects with very low (<1 mg/l) initial plasma levels and only four of the others showed rises greater than 5 mg/l. All elderly patients excreted only trivial amounts of vitamin C in the urine (1% mean; range 0.5–2%) in the first 6 h.

The results suggest that absorption and excretion of vitamin C in the elderly patients are very different from those in healthy young subjects, and that the ascorbic acid tolerance test may be used to assess the vitamin C status in the elderly.

Informed consent was obtained from all subjects and the project was approved by the local ethical committee. We thank Dr R. Boyd for permission to study patients under his care.

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Current social factors and the growth of pre-school children. By Peter T.
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Evidence from a recent representative study of 1- and 2-year-old children in
Britain confirms a strong association between such factors as parental unemploy-
ment and child growth.

The survey, conducted by this department for the Department of Health and
Social Security in 1981–82, involved 9765 subjects drawn from 103 local
authorities in England, Wales and Scotland. Samples were drawn using the Child
Benefit Register and the authorities were selected on socio-economic criteria using
a classification prepared by the Office of Population, Census and Surveys (Weber &
Craig, 1978). Socio-economic interviews were conducted in the homes of the
subjects and length, weight, arm muscle circumference, triceps and subscapular
skinfold measurements were made. Where possible the height of the natural
mothers was also collected.

Analysis of attained length shows the usual strong association between
biological variables such as birth weight, gestation, mother's height and parity and
difference in growth. However, the proportion of the variance attributable to social
variables is dominated by the effect of unemployment and its associated factors.
The contribution of unemployment as a variable does not disappear when birth
weight, gestation, mother's height, father's social class and geographic region of
residence are controlled in the analysis. Further study of these data is being
undertaken and the relationship between parental unemployment, family income,
child growth and morbidity investigated.

*Effect of social class and unemployment on length of 1- to 2-year-old children
allowing for short gestation, birth weight and mother's height*

(Analysis of variance. Values in parentheses are all below 20 and the means are considered
unreliable)

		Length of child (m)							
		1-year-olds				2-year-olds			
		Boys		Girls		Boys		Girls	
Social class	Employment status§	Mean	n	Mean	n	Mean	n	Mean	n
IIInm	E	0·757	254	0·744	237	0·874	239	0·856	239
	S-UE	0·759	(7)	0·752	(4)	0·839	(7)	0·858	(5)
	UE	0·738	(15)	0·735	(5)	0·898	(7)	0·857	(12)
IIIm	E	0·756	781	0·741	786	0·872	776	0·858	751
	S-UE	0·752	58	0·737	52	0·860	44	0·849	56
	UE	0·754	116	0·739	112	0·861*	98	0·851	100
IV-V	E	0·758	258	0·741	253	0·873	212	0·860	231
	S-UE	0·752	29	0·747	(19)	0·872	23	0·832	(14)
	UE	0·750	66	0·735	78	0·853†	67	0·845††	59

Significantly different from IIIm E: *P=0·05.
Significantly different from IV-V E: †P=0·05, ††P=0·01.
§At family interview: E, 'breadwinner' currently employed; S-UE, 'breadwinner' unemployed
under 6 months; UE, 'breadwinner' unemployed over 6 months.

Webber, R. & Craig, J. (1978). *Socio-economic Classification of Local Authority Areas.
Studies on Medical and Population Subjects* no. 35. London: HM Stationery Office.

Urine 24 h nitrogen excretion as an independent measure of the habitual dietary protein intake in individuals. By SHEILA BINGHAM and J. H. CUMMINGS, *MRC Dunn Clinical Nutrition Centre, Addenbrooke's Hospital, Trumpington Street, Cambridge CB2 1QE*

It has been suggested that 24 h urinary excretion of nitrogen be used as an independent index of protein intake to validate dietary methods (Isaksson, 1980). To assess this in individuals, eight healthy subjects (five men, three women, body-weight 45–95 kg) were studied for 24 d whilst consuming their usual, varying diets in a metabolic unit, and continuing their working and social lives within the protocol limits. Throughout the study daily duplicates of all food eaten and continuous 24 h collections of urine and faeces were made for N analysis by the Kjeldahl method. The completeness of collections was verified with the use of continuous markers, *p*-aminobenzoic acid for urine (Bingham & Cummings, 1983) and radio-opaque pellets for faeces (Branch & Cummings, 1978). Losses of N in blood and from the skin were also measured (Calloway *et al.* 1971). Results are shown in the Table.

Subject ...	JC	AB	MB	PM	CM	RB	CS	SF
Energy intake* (MJ/d)	11·2	18·2	14·4	12·9	11·8	8·5	9·2	11·8
Nitrogen intake (g/d)	14·34	20·91	21·29	17·31	14·86	11·61	12·87	14·03
Nitrogen output (g/d)								
Urine	11·68	16·38	17·16	13·71	12·34	9·67	10·39	11·46
Faeces	1·74	2·45	2·24	2·51	2·57	1·28	1·98	1·82
Blood	0·05	0·05	0·05	0·06	0·05	0·12	0·17	0·11
Skin	0·36	0·77	0·47	0·37	0·36	0·25	0·29	0·42
Coefficients of variation (%)								
Diet N	26	18	24	20	22	14	18	22
Urine N	14	11	18	10	13	10	13	14

*Calculated from food tables (Paul & Southgate, 1978; Wiles *et al.* 1980).

Average energy intake from protein was a constant percentage of total habitual energy intake, 14 (SD 1) MJ %, and urine N was a constant percentage of dietary intake, 81 (SD 2) %. However, within person coefficients of variation in dietary intake ranged from 14 to 26%, so that an average of 18 d were required to estimate the habitual mean to within a SE of 5%. Urine N excretion was more constant. Complete collections for an average of 8 d were required to be within a SE of 5% of the overall mean; these were sufficient to estimate urine N to be within 81 (SD 5) % of the habitual dietary N intake of individuals.

Bingham, S. A. & Cummings, J. H. (1983). *Clinical Science* **64,** 629–635.
Branch, W. J. & Cummings, J. H. (1978). *Gut* **19,** 371–376.
Calloway, D. H., O'Dell, A. C. F. & Margen, F. (1971). *Journal of Nutrition* **101,** 775–786.
Isaksson, B. (1980). *American Journal of Clinical Nutrition* **33,** 4–12.
Paul, A. A. & Southgate, D. A. T. (1978). *McCance & Widdowson's, 'The Composition of Foods'*, MRC Special Report no. 297. London: H.M. Stationery Office.
Wiles, S. J., Nettleton, P. A., Black, A. E. & Paul, A. A. (1980). *Journal of Human Nutrition* **34,** 189–223.

Reduced nitrogen excretion with an anabolic steroid in the post-operative period. By O. J. GARDEN, S. L. BLAMEY, A. SHENKIN and D. C. CARTER, *University Departments of Surgery and Biochemistry, Royal Infirmary, Glasgow G4 0SF*

Although anabolic steroids have been shown to improve nitrogen balance in the postoperative period, their use has been restricted to male patients because of their androgenic effect (Johnston & Chenneour, 1963). This study assesses the effect of an anabolic steroid (stanozolol) with a low androgenic effect on both male and female patients undergoing abdominal surgery.

Thirty-six patients, stratified to include equal numbers of males and females, were randomly allocated to receive a single preoperative intramuscular injection of 50 mg stanozolol or placebo. Both groups were well matched with respect to age, weight and type of operation. During the first four postoperative days, patients received only intravenous fluids and water by mouth. Urine collections (24 h) were made for total urine N, 3-methyl histidine and creatinine.

The Table shows the 4 d mean N excretion (gN/d). The significance of differences was analysed by Student's t test.

	Control		Stanozolol		Significance
	Mean	SD	Mean	SD	($P<$)
Male	13·0	3·7	9·6	2·8	0·05
Female	8·8	3·5	6·3	2·2	Not significant
Over-all	10·9	4·1	8·0	3·0	0·02

The mean (and SD) daily excretion of 3-methyl histidine of 30·3 (8·0) µmol/mmol creatinine in the control group was not significantly different from 30·8 (9·6) µmol/mmol creatinine in the stanozolol group.

In the female patients, cumulative fluid balance over the 4-d period was significantly more positive in the stanozolol group (3577 (1475) ml) than in the control group (1167 (1683) ml, $P<0·05$). There was no significant difference between groups in terms of fluid balance in male patients (stanozolol 3284 (2191) ml, control 2791 (2030) ml). There was no significant difference in serum urea or creatinine between the groups.

Improved N balance in the absence of a measurable change in muscle catabolism suggests that stanozolol acts by improving protein synthesis in the early period following surgery. This effect appears to be more marked in male patients and is not accompanied by the fluid retention observed in female patients.

Johnston, I. D. A. & Chenneour, R. (1963). *British Journal of Surgery* **50**, 924–928.

Double metabolic balances on low-birth-weight infants given formulae of differing composition. By G.-S. HARRI PERSAUD, J. B. MORGAN, R. F. GRIMBLE and C. J. ROLLES, *Departments of Child Health and Nutrition, Faculty of Medicine, University of Southampton, Southampton SO9 5NH*

The comparative nutritional merits of two infant milk formulae Ready-to-Feed Premium Babyfood® (Cow & Gate Ltd) and Prematalac® (Cow & Gate Ltd) were examined. Prematalac is modified to meet more closely the unique requirements of the low-birth-weight infant (Fomon *et al.* 1977). Nine infants (mean (and SD) birth weight 1332 (478) g, birth age 31 (4) weeks) were enrolled for two metabolic balance periods in a cross-over experimental design. At the onset, infants were randomly allocated one of two milks. Daily milk intakes were precisely measured. Total faecal and urine collections were made over corresponding periods. The faeces relating to the first and last feeds were identified with carmine in these milks. All samples were analysed for energy by bomb calorimetry and for total nitrogen by the Kjeldahl method. Various anthropometric measurements were collected at the start and end of each 4 d balance.

	Premium		Prematalac		Significance
	Mean	SD	Mean	SD	($P\leqslant$)
Incremental weights (g/d)	19	11	46	20	0·005
Incremental length (mm/balance)	5	5	5	6	NS
Energy intake (kJ/kg per d)	527	59	745	117	0·005
Faecal energy (kJ/kg per d)	40	25	59	29	0·01
% Digestibility	92	4	92	5	NS
N intake (mg/kg per d)	569	53	721	91	0·005
Faecal N (mg/kg per d)	85	42	80	40	NS
Urinary N (mg/kg per d)	187	45	168	53	NS
% N retained	52	5	66	5	0·005

NS, not significant.

All infants received more energy and protein/kg body-weight per d on Prematalac compared with Premium. Incremental weight, though satisfactory for both milks, was greater when Prematalac was given compared with Premium. No differences in length gain were detected. Mean percentage digestibility of energy intake was similar for both milks. Infants retained more N when given Prematalac compared with Premium. Neither milk was considered ideal, and in these experiments it appeared that the standard formula did not support the potential for gain in lean body tissue of the premature infants.

Fomon, S. J., Ziegler, E. E. & Vazquez, H. D. (1977). *American Journal of Diseases in Childhood* **131**, 463–467.

Gut hormones in malnourished children with protracted diarrhoea. By A. M. TOMKINS, *Medical Research Council Laboratories, Fajara, The Gambia* and H. PATEL, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT* and S. R. BLOOM, *Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 0HS*

There are multiple factors which contribute to the diarrhoea which is a major complicating feature among children with protein-energy malnutrition (PEM). However, persistent bacterial colonization of the upper intestine is a frequent finding. Intestinal stasis facilitates bacterial colonization; this may occur as a result of the effect of gut hormones released by mucosal cells which have inhibitory or stimulatory effects on secretion and motility.

Levels of gut hormones (pmol/l) were measured in plasma samples from eighteen malnourished Gambian children (marasmus, marasmic-kwashiorkor or kwashiorkor) with protracted diarrhoea (PD/PEM), in seven malnourished children without diarrhoea (PEM) and in eleven well-nourished children without diarrhoea (controls). Samples were taken 60 min after a formula feed. Mean enteroglucagon levels were higher in PD/PEM (630 (SE 43)) than in PEM (213 (SE 53)) or in controls (217 (SE 23)) ($P<0.001$). Motilin levels were higher in PD/PEM (212 (SE 14)) than in controls (149 (SE 19)) ($P<0.02$). Gastrin levels were higher in both PD/PEM (168 (SE 30)) ($P<0.001$) and PEM (86 (SE 15)) ($P<0.02$) than in controls (43 (SE 6)); the differences between PD/PEM were significant ($P<0.05$). Levels of neurotensin, vasoactive intestinal peptide, cholecystokinin and insulin were not significantly different. Following successful treatment of the diarrhoea in five children there was a reduction in levels of enteroglucagon ($P<0.02$) and gastrin ($P<0.01$).

Raised gastrin levels suggest that a gastric mucosal lesion is a common feature of these malnourished children especially in those with PD/PEM. Raised enteroglucagon levels suggest that an intestinal mucosal lesion is also present, particularly in those with PD/PEM. We suggest that enteroglucagon delays transit through the small intestine sufficiently to facilitate bacterial colonization which is a major cause of diarrhoea in malnourished children.

Changes in the biliary lipids in bile obtained from gall-bladders with and without cholesterol gallstones. By M. S. SIAN, *Professorial Department of Surgery, Charing Cross Hospital Medical School, London W6 8RF*

Cholesterol gallstone disease is a common condition and there is evidence that the incidence is increasing. Alterations in the bile salt composition of bile have been implicated in the aetiology of this condition (Bouchier, 1979) but few studies related to these changes have been reported. This study was undertaken to investigate the changes in bile salt composition and biliary lipids (cholesterol and phospholipids) in gall-bladder bile of patients suffering from cholesterol gallstones.

Gall-bladder bile from gallstone patients (gallstone bile) was obtained at cholecystectomy; non-gallstone (control) bile was obtained at post-mortem within 48 h of death from gall-bladders of subjects without biliary disease.

Bile salts were estimated using high-pressure liquid chromatography (HPLC) methods developed in this laboratory (Sian & Rains, 1979). Biliary cholesterol was measured using gas chromatography and the phospholipid content of bile estimated by spectrophotometry.

Total bile salt concentration in gallstone bile was significantly lower than in non-gallstone bile. Qualitatively, the types of bile salts in the two groups were similar. However, the percentage of deoxycholic acid was greater in gallstone bile (24% in gallstone bile v. 14% in non-gallstone bile, $P < 0.001$). The percentage of cholic acid was unchanged but percentage of chenodeoxycholic was lower in gallstone patients (31% in gallstone bile v. 40% in non-gallstone bile, $P < 0.001$). Marked differences were found in the proportions of glycine and taurine bile salts in the two groups. The concentration of glycodeoxycholic was increased but that of taurochenodeoxycholic was reduced in gallstone bile. The results are summarized in the Table.

Composition of gall-bladder bile

	Gallstone bile (n 16)		Non-gallstone bile (n 16)		Statistical significance
	Mean	SE	Mean	SE	$P <$
Bile salts (mmol/l)	54·7	15·4	94·7	27·3	0·001
Phospholipids (mmol/l)	29·6	14·7	35·1	10·8	0·05
Cholesterol (mmol/l)	12·2	6·1	5·7	4·0	0·001
Total lipids (mmol/l)	86·5	28·1	135·5	28·9	0·001
Ratios					
Bile salts:cholesterol	5:1		17:1		
Phospholipids:cholesterol	2:1		6:1		
$\dfrac{\text{Bile salts + phospholipids}}{\text{Cholesterol}}$	7:1		23:1		

Increased deoxycholic acid and a decrease in the proportion of chenodeoxycholic acid in gallstone bile provides further evidence that this type of bile favours cholesterol gallstone formation.

Bouchier, I. A. D. (1979). *British Journal of Clinical Practice* **33**, 37–42.
Sian, M. S. & Rains, A. J. H. (1979). *Clinica Chimica Acta* **98**, 243–252.

Longitudinal study of food habits of Nigerian students in Aberdeen.

By GILLIAN M. LOCKIE, *Robert Gordon's Institute of Technology, School of Nutritional Science, Kepplestone Premises, Queen's Road, Aberdeen AB9 2PG*

Food habits of British students have been investigated by Stordy (1973) and Fieldhouse (1980). Al-Mokhalalati (1982) carried out a survey of Sudanese students' food choice. The results of an investigation into the effect of length of stay on the food habits of Nigerian students are reported here.

Six students completed two 7-d diaries and provided qualitative data about food consumption at each meal. Information about snacks was also recorded. An interval of at least 1 year separated the two surveys. Five of the students were married, although at the time of the first investigation, only one of the subject's wives was living in Aberdeen. The Nigerians lived in self-catering accommodation.

The Wilcoxon test for paired comparisons showed that the total frequency of consumption of peas, beans and sweetcorn was significantly lower in week 2 ($\alpha = 0.01$ two sided test). The total frequency of consumption of apples, bananas and pineapple, biscuits and citrus fruits was lower in week 2 but the decrease was not statistically significant. Three of the students did not eat citrus fruits in week 1 or week 2. Sausages and fish and chips were eaten more frequently in week 2 but this change in food choice was not statistically significant.

During week 2 there was a greater intake of traditional Nigerian foods at lunch-time and a decrease in their consumption in the middle of the afternoon. The increase in rice, ground rice or semolina at lunch was significant as was the fall in their consumption mid-afternoon ($P<0.05$, two tailed test in both cases). The increase in soup or stew consumption at lunch was not significant, although the fall in intake of this dish mid-afternoon was significant ($P<0.01$, two tailed test). The decreased intake of white bread, sausages and beefburgers at lunch in week 2 was not statistically significant, although the fall in egg consumption was significant ($P<0.05$, two tailed test).

These results suggest that length of stay in Aberdeen had little effect on the food selected by the Nigerian students surveyed and confirm the findings of Al-Mokhalalati (1982) on Sudanese students.

Al-Mokhalalati, J. (1982). *Proceedings of the Nutrition Society* **41**, 239–242.
Fieldhouse, P. (1980). *Nutrition and Food Science* no. 63, pp. 12–14.
Stordy, B. J. (1973). *Nutrition* **27**, 262–266.

Breath methane levels and intestinal methanogenesis among rural Nigerians on a local diet. By B. S. DRASAR and A. M. TOMKINS, *London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT* and H. WIGGINS, *Dunn Clinical Nutrition Centre, Cambridge CB2 1QE* and M. HUDSON, *Public Health Laboratory Service, Porton Down, Salisbury SP4 0JG*

Methane is detectable in the breath in a varying proportion of subjects in different populations. Breath methane is absent in the germ-free state. Manipulation of dietary carbohydrate intakes in individual subjects seems not to affect the amount of methane excreted in the breath. In view of the absence of information on subjects habitually eating a high-fibre diet, we measured breath methane levels in a rural Nigerian population on whom faecal bacteriology was performed.

The population had a predominantly sorghum- and rice-based diet. None had a recent history of diarrhoea or had received antibiotics in the preceding month. Their nutritional status and intestinal flora have been described previously. Breath methane was measured in samples of terminal alveolar air obtained using a per-nasal cannula or mouthpiece. Faecal samples were injected into a modified methanogen-enrichment broth (BG-6) contained in serum-stoppered vials designed for use with an automated head space gas chromatograph. Uninoculated BG-6 medium contained less than 2 mg methane/l. The percentage of faecal samples in which more than 3 mg/l occurred is shown in Table 1. Breath methane was recorded as positive if levels of more than 1 mg/l above atmosphere levels were detected (Table 2).

Table 1. *Number of subjects in which bacterial methane production occurred in vitro (percentage in parentheses)*

Dilution of faecal sample ...	10^{-2}	10^{-4}	10^{-6}
Young children (n 6)	6/6 (100)	4/6 (67)	3/6 (50)
Older children (n 7)	6/7 (86)	6/7 (86)	6/7 (86)
Adults (n 36)	33/36 (92)	29/36 (81)	20/36 (56)

Table 2. *Percentage of subjects with methane in breath*

	Total	Distribution of subjects according to breath methane concentration (mg/l)						
		None	10	11–20	21–30	31–40	41–50	50
Young children (n 49)	8	92	4	0	2	2	0	0
Older children (n 47)	40	60	24	4	8	4	0	0
Adults (n 159)	77	22	24	18	14	6	8	8

Breath methane excretion was least frequently detected among young children and most commonly detected in adults. Methane production by faecal samples was similarly distributed throughout the age groups and there was no direct correlation between the amount of methane produced by incubation of a faecal sample and the amount of methane detected in the breath. We suggest that intestinal factors such as length, content and transit time are necessary for intestinal methanogenesis to occur in sufficient quantity to be detected in the breath.

Effects of taste sensitivity and preference on salt intake. By R. Shepherd,
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Lane, Norwich NR4 7UA, UK*

High salt intake has been associated with hypertension (Tobian, 1979), and
differences between individuals in salt intake may be related to taste sensitivity
and preference levels for salt in foods (Contreras, 1978).

Total salt intake was estimated from urinary excretion of sodium over seven
consecutive days in thirty-three normal subjects, and table and cooking salt usage
were measured using weighed salt pots. Sensitivity to salt taste was assessed for
thirty-one of the subjects using a 7-category rating scale of intensity for saltiness
of five salt concentrations in water, bread and mashed potato. The measures of
sensitivity used were the rate of increase of perceived intensity with increasing
concentration (i.e. the slope of the psychophysical function), and the intercept for
this line. Preferences were measured for the bread and mashed potato using a
9-point hedonic rating scale to determine the maximally preferred concentration
for each subject.

No differences were found in the taste measures between high- and low-intake
groups divided on the basis of either total salt intake or total intake divided by
body-weight. However, when subjects were divided according to table salt usage
those with high usage showed lower sensitivity in terms of the slope of the
psychophysical function for the mashed potato ($F_{(1,27)} = 6.4$, $P<0.05$), and
showed a preference for higher concentrations in the bread ($F_{(1,27)} = 4.7$,
$P<0.05$; preferred concentrations for low intake group $= 6.44$ g Na/kg, for high
intake group $= 8.57$ g Na/kg).

The major percentage of total intake (84%) was from salt in foods rather than
added during cooking or at the table. Intake from table salt is entirely under
voluntary control of the individual and so would be expected to be related to
sensitivity and preference. However, most foods are not sold with a choice of salt
levels (e.g. a normal product and a low-salt product), and so the only control the
individual has over intake from this source is the amount of food consumed and
choice between different types of food. Since this choice may be influenced by
many other factors (e.g. price, convenience, tastes other than saltiness) it is not
surprising that sensitivity to and preference for salt are not the major determining
factors of intake from this source, and hence of total intake.

Contreras, R. J. (1978). *American Journal of Clinical Nutrition* **31**, 1088–1097.
Tobian, L. (1979). *American Journal of Clinical Nutrition* **32**, 2739–2748.

Assessment of nutritional status of college students in the Dublin area. By
M. KENNEDY[1], M. McCORMACK[2], B. A. RYAN[1] and P. M. MATHIAS[1], [1]*Dublin Institute of Technology, Kevin Street, Dublin* 8, *Irish Republic* and [2]*County Hospital, Mullingar, County Westmeath, Irish Republic*

Fifty-eight students (thirty males and twenty-eight females) were investigated. Their ages ranged from 17 to 23 years. Heights and weights were measured, and readings of skinfold thickness were made at four body sites (biceps, triceps, subscapular and suprailiac) using a Holtain skinfold caliper. Body mass index (BMI; weight/height^2) was calculated and percentage body fat estimated from the sum of the skinfold thicknesses using the equations of Durnin & Womersley (1967). One male and one female were 20% above the upper weight limit for their height and could thus be classed as 'obese'. They had the highest BMIs in their groups (28·8 and 26·5 respectively) and high values for percentage body fat (26 and 30% respectively). There was a significant correlation between BMI and percentage body fat in both males (r 0·58, $P<$0·01) and females (r 0·53, $P<$0·01). These results confirm that the index weight:height^2 is a satisfactory index of relative weight in individuals.

Mean daily nutrient intakes were assessed from 7-d dietary diaries kept by each student, using values in McCance and Widdowson's food tables (Paul & Southgate, 1978). The average daily energy intake in males was 11·5 MJ. As there was little obesity in this group, this result suggests that the Irish recommended daily nutrient intake (RDNI) of 10·5 MJ/d (Department of Health, 1983) may be set too low. The average intake in females was 8·2 MJ/d (RDNI 9·0 MJ/d); a dietary questionnaire indicated that none were on reducing diets at the time of the study. There was no significant correlation between BMI and energy intakes in either group. The only nutrient intakes which fell appreciably below Irish RDNIs were folic acid in males (60% RDNI) and females (54% RDNI), and iron in females (78% RDNI), although blood haemoglobin (Hb) and red cell folates (RCF) were within the normal range in both groups. There was no significant correlation between dietary intake of Fe and Hb nor between intake of folic acid and RCF in either males or females.

These results suggest that the low intakes of Fe and folate may not be habitual in these subjects, or that RDNIs may be set too high. However, these results must be considered in context with regard to the importance of nutrient composition (especially with respect to Fe and folate) of pre- and periconceptional diets of women of child-bearing age (Schorah *et al.* 1983).

Department of Health (1983). *Recommended Daily Nutrient Intakes of the Republic of Ireland.* Dublin: Stationery Office.
Durnin, J. V. G. A. & Womersley, J. (1967). *British Journal of Nutrition* **21**, 681–689.
Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's 'The Composition of Foods'.* London: H.M. Stationery Office.
Schorah, C. J., Wild, J., Hartley, R., Sheppard, S. & Smithells, R. W. (1983). *British Journal of Nutrition* **49**, 203–211.

The effect of cooked legumes on mucosal cell turnover in the rat. By
SANOJA S. SANDARADURA and A. E. BENDER, *Department of Nutrition,
Queen Elizabeth College, University of London, London W8 7AH*

The low digestibility of legume protein, 70–80%, has been well documented.
Bender & Mohammadiha (1981) reported that giving cooked legumes to rats
resulted in an elevated faecal excretion of DNA compared with rats given a casein
diet, and concluded that this probably arose from increased turnover of mucosal
cells of the intestine rather than from bacteria.

However, Fairweather-Tait *et al.* (1983) concluded, from experiments in which
^{3}H-labelled thymidine was given, that beans caused only a small increase in the
turnover of mucosal cells.

In the present study the rate of cell turnover has been measured by counting cell
mitoses after treatment with cochicine, which arrests cell division at metaphase.

Three groups each of five weanling rats were fed *ad lib.* with diets containing
cooked, whole white kidney beans (*Phaseolus vulgaris*), cooked dehusked beans or
casein at 200 g protein/kg diet. After 10 d the animals were injected intra-
peritoneally with a saline solution (9 g sodium chloride/l) of colchicine (1 mg/kg
body-weight) and killed exactly 2 h later. Two pieces of intestine, each 20 mm in
length, were cut starting 200 mm from the pylorus, washed, slit open and fixed in
formyl-saline before sectioning and stained with Feulgen's reagent.

Cells arrested at metaphase were counted in forty crypts in each animal.

Diet ...	Whole bean		Dehusked bean		Casein	
	Mean	SEM	Mean	SEM	Mean	SEM
Faecal N (mg/g food)	7·18**	0·21	7·84**	0·37	4·01	0·17
Faecal DNA (mg/g food)	1·189**	0·098	0·985**	0·987	0·545	0·027
Metaphases/crypt	28**	0·7	26**	0·7	17	0·5

Significantly different from control: **$P<0.01$.

Rats fed on whole or dehusked beans excreted nearly twice as much nitrogen
and twice as much DNA in the faeces as those fed on casein diets, and the number
of cells that had undergone division during the 2 h before killing were nearly
double those on the casein diet (see Table). This suggests that the increased faecal
DNA did not arise from intestinal bacteria nor from the diet but from increased
turnover of mucosal cells, and casts doubt on the validity of results regarding the
'true' N digestibility of legumes.

Bender, A. E. & Mohammadiha, H. (1981). *Proceedings of the Nutrition Society* **40**, 66A.
Fairweather-Tait, S. J., Gee, J. M. & Johnson, I. T. (1983). *British Journal of Nutrition* **49**,
303–312.

Nutritional and immunological assessment of patients with anorexia nervosa. By PAULINE S. DOWD, J. KELLEHER, B. E. WALKER and P. J. GUILLOU, *Departments of Surgery and Medicine, Clinical Sciences Building, St. James's University Hospital, Leeds LS9 7TF*

It is now recognized that there exists a relationship between nutritional status and immunological function (Chandra, 1980). In an attempt to assess this relationship in patients with anorexia nervosa, various nutritional indices and two aspects of cellular immunity (T-cell function and natural killer cell activity) were measured. Anthropometric measurements were found to be depressed, as were the serum levels of zinc ($P<0.01$), copper ($P<0.01$) and ceruloplasmin ($P<0.05$) compared with a control group of similar age range. However, the serum levels of albumin, transferrin, vitamin A and leucocyte vitamin C were generally found to be within normal limits.

Lymphocyte transformation was measured in response to stimulation with the mitogens concanavalin A (Con A) and phytohaemagglutinin (PHA). Responses were normal in all of the seven patients assessed with the exception of one who showed a depressed response to the mitogen Con A. The natural killer cell activity of peripheral blood lymphocytes was measured in a 4 h ^{51}chromium release assay against the target cell line K562. There was no significant difference between ten anorexia nervosa patients and the control group. The results of nutritional and immunological investigations are summarized in the Table.

	Anorexics			Controls		
	n	Mean	SD	n	Mean	SD
Albumin (g/l)	18	36.1	5.0	20	38.2	2.7
Transferrin (g/l)	18	2.5	0.5	20	2.4	0.5
Vitamin A (µg/l)	18	1090	640	20	950	180
Vitamin C (µg/10^8 white blood cells	17	25.8	11.6	20	26.6	8.7
Zinc (µmol/l)	16	12.7**	2.2	20	14.8	2.0
Copper (µmol/l)	16	15.6**	4.5	20	18.7	2.6
Ceruloplasmin (mg/l)	16	323*	100	20	393	65
Lymphocyte proliferation to:						
Con A (counts/min × 10^{-3})	7	146	64	6	147	60
PHA (counts/min × 10^{-3})	7	156	47	6	146	57
Natural killer cell activity (% ^{51}Cr release)	10	34.4	13.0	8	26.8	8.6

Significantly different from controls: *$P<0.05$, **$P<0.01$.

It is suggested that the relatively normal protein and vitamin levels in patients with anorexia nervosa is responsible for maintaining their apparently normal cellular immune function.

Chandra, R. K. (1980). *Immunology of Nutritional Disorders. Current Topics in Immunology, Series no. 12.* London: Edward Arnold.