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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Ninety-ninth Meeting of the Nutrition Society was held in the Edward Lewis Lecture Theatre of the Middlesex Hospital Medical School, Cleveland Street, London W1P 7PN, on Thursday, 2 December, 1976, *when the following papers were read:*

https://doi.org/10.1079/PNS19770020 Published online by Cambridge University Press

Reduced cytochrome *c* as an electron donor for microsomal mixed-function oxidase reactions. By K. W. J. WAHLE and N. T. DAVIES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Microsomes from certain animal tissues contain an electron-transport chain (ETC), which is involved in various mixed-function oxidase reactions including ω-hydroxylation and desaturation of fatty acids (Gunsalus *et al.* 1975). The possibility of utilizing the oxidation of NADH and/or NADPH for a simple, spectrophotometric assay of reaction rates was precluded by the rapid transfer of electrons to cytochrome b_5 which apparently acts as an 'electron reservoir'.

Oxidation of reduced cytochrome *c* can be employed in the spectrophotometric determination of cytochrome oxidase activity (Mills & Dalgarno, 1970). The commercial availability of this cytochrome and its ready reduction by chemical means led to its being considered as an electron donor for the microsomal ETC in a spectrophotometric assay for mixed-function oxidase reactions.

Comparative investigations showed that reduced cytochrome *c* was as effective as NADH or NADPH in supplying electrons for both Δ9-desaturation and ω-hydroxylation of fatty acids.

The oxidation rate of reduced cytochrome *c* in the presence of liver microsomes was dependent on the addition of fatty acyl-CoA derivatives and could be determined spectrophotometrically at 550 nM. The reaction had the properties of a mixed-function oxidase in that it required molecular oxygen and was inhibited by CO, azide and CN^- (Gunsalus *et al.* 1975).

Hepatic microsomal Δ9-desaturase activity, determined by a standard assay procedure (Wahle, 1974), was reduced in starved rats and was greater in starved–refed rats than in control animals. However, stearoyl-CoA-dependent oxidation of reduced cytochrome *c* was inversely related to observations using the standard desaturase assay, being greatest in microsomes from starved rats and least in those from fed animals. This pattern of activity is characteristic of ω-hydroxylation of fatty acids (Björkhem, 1973) and suggests that reduced cytochrome *c* can provide electrons preferentially to cytochrome P_{450} under the conditions of the spectrophotometric assay. Subsequent investigations into the effect of dietary copper deficiency or copper-chelators in vitro on the microsomal ω-hydroxylation of fatty acids were carried out using this spectrophotometric technique (Wahle *et al.*, 1977).

Björkhem, I. (1973). *Eur. J. Biochem.* **40**, 415.
Gunsalus, I. C., Pederson, T. C. & Sligar, S. G. (1975). *Ann. Rev. Biochem.* **44**, 377.
Mills, C. F. & Dalgarno, A. C. (1970). In *Trace Element Metabolism in Animals*, p. 456. Edinburgh and London: Livingstone.
Wahle, K. W. J. (1974). *Comp. Biochem. Physiol.* **48B**, 87.
Wahle, K. W. J., Davies, N. T. & Paterson, S. M. (1977). *Proc. Nutr. Soc.* **36**, 30A.

ω-Hydroxylation of fatty acids by microsomal preparations from rat and sheep tissues. By K. W. J. WAHLE, N. T. DAVIES and SANDRA M. PATERSON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

ω-Hydroxylation of fatty acids by rat liver and kidney is a mixed-function oxidase reaction which requires a microsomal electron-transport chain (ETC) and cytochrome P_{450} (Wada *et al.* 1968; Ellin *et al.* 1972). The reaction is greatly enhanced in liver preparations from starved, fat-fed or diabetic rats (Björkhein, 1973). ω-Hydroxy fatty acids can be further oxidized to dicarboxylic acids (Verkade, 1938; Wada *et al.* 1968) which on β-oxidation give rise to succinate which can enter the tricarboxylic acid cycle and be utilized for acetate oxidation or gluconeogenesis (Verkade, 1938; Wada *et al.* 1971). Glucose synthesis from fatty acids might have greater physiological significance in ruminants than in simple-stomached animals, because glucose availability in ruminants is at a premium, particularly in twin-bearing ewes and high-yielding dairy cows, and glucose requirements are largely met by gluconeogenesis (Ballard *et al.* 1969). Aspects of the ω-hydroxylase activity of sheep and rat liver microsomes were therefore investigated.

Addition of particle-free supernatant or NAD^+ and alcohol dehydrogenase to microsomal incubations had little effect on the ω-hydroxylation of lauric and palmitic acid by sheep or rat liver microsomes. Lauric acid was hydroxylated to a greater extent than palmitic acid by both species and the degree of hydroxylation of lauric acid was greater in rat than in sheep preparations.

Induction of cytochrome P_{450} in rat liver by phenobarbital (Björkhem & Danielsson, 1970) had little effect on microsomal ω-hydroxylation of lauric acid.

Liver microsomes from copper-deficient rats had lower ω-hydroxylase activity than those from control animals. Studies in vitro with copper-chelators (e.g. bathocuproine, cuprizone) using either a standard assay or a spectrophotometric assay (Wahle & Davies, 1977) indicated a catalytic involvement of copper in the reaction. It appears therefore that copper is involved in two ETC-dependent mixed-function oxidase reactions namely, ω-hydroxylation and Δ9-desaturation (Wahle & Davies, 1975) of fatty acids.

The results also show that the ω-hydroxylation system is active in microsomes from sheep liver and that its properties are similar to those of the rat system.

Ballard, F. J., Hanson, R. W. & Kronfeld, D. S. (1969). *Fed. Proc.* **28**, 218.
Björkhem, I. & Danielsson, H. (1970). *Eur. J. Biochem.* **17**, 450.
Björkhem, I. (1973). *Eur. J. Biochem.* **40**, 415.
Ellin, A., Jakobsson, S. V., Schenkman, J. B. & Orrenius, S. (1972). *Arch. Biochem. Biophys.* **150**, 64.
Verkade, P. E. (1938). *Chemy Ind.* **57**, 704.
Wada, F., Shibata, H., Goto, M. & Sakamoto, Y. (1968). *Biochim. biophys. Acta* **162**, 578.
Wada, F., Usami, M., Goto, M. & Hayaishi, T. (1971). *J. Biochem. Tokyo.* **70**, 1065.
Wahle, K. W. J. & Davies, N. T. (1975). *Br. J. Nutr.* **34**, 105.
Wahle, K. W. J. & Davies, N. T. (1977). *Proc. Nutr. Soc.* **36**, 29A.

Mechanism for regulating nitrogen balance in man. By P. V. SUKHATME, *Maharashtra Association for Cultivation of Science, Poona-4, India*

There is considerable evidence to suggest that man possesses a physiological regulatory mechanism which serves to maintain a near constant body-weight (energy balance) for extended periods. However, there is no such evidence to suggest that a comparable mechanism exists for other nutrients, particularly nitrogen. In consequence, day-to-day fluctuations in N balance in man are held to be random in character, arising from errors of measurement, and these, as such, can be eliminated by averaging the balance over a sufficiently long period, 3, 5, or 7 d as necessary. This has led to the assumption that man's requirement for N, while varying from individual to individual of the same age–sex group, is essentially constant over time in the same individual. This assumption is, however, not borne out by the results available in the literature. It is found that the successive values of N, even when they are averaged over several days, behave differently from the random series. The successive values are found to fluctuate considerably from day-to-day with output increasing sharply from 1 to 3 d and diminishing gradually over 4 to 8 d, showing that they are not random, but are correlated. Actual calculations show that the values of auto correlations of successive orders fall rapidly, showing that the N balance on any day is most influenced by the value of the balance immediately preceding it and that the influence of the preceding value becomes less and less as the time lag increases, as one would expect in a biological phenomenon regulated through time. Detailed results are presented in the paper. It is concluded that the mechanism regulating N balance in man is probabilistic in kind represented by the Marcoff series remaining stationary through time and not deterministic as assumed in the nutrition literature. The implications of this finding for defining deficiency and evaluating its incidence in the population are spelt out.

The influence of the addition of small amounts of amino acid and energy on nitrogen balance in total starvation in obese patients. By ANJE WIERSINGA and A. VAN LOENEN, *University Hospital, Department of Medicine, Utrecht, Netherlands*

Prolonged total starvation in gross obesity is used to reduce total body fat. Loss of lean body mass is unwanted. Baird *et al.* (1974) obtained, in five obese subjects during starvation, nitrogen balance with a daily intake of 30 g amino acids (AA) and also with 15 g AA/d, when 30–45 g carbohydrate/d was added.

The purpose of our study was to determine whether N balance in obese subjects during starvation on an intake of 20 g AA/d improved with the addition of 20 g glucose/d or due to the addition of 336 J/d, either as glucose or as oil.

Three patients were studied in the metabolic ward (patient G twice) from 6 to 10 weeks. All had a normal 50 g glucose tolerance test, no detectable endocrine or other disorder. They were physically active. Water, black coffee and tea were allowed. Vitamin and mineral supplements were given. Each experimental period (A, S, F and O) lasted 14 d. In periods A, S, and F 20 g/d L-amino acids were given, half essential and half non-essential, corresponding with 2·56 g N/d or 35·9 g N/14 d. In period S 20 g glucose/d and in period F 9 g sunflower oil/d were added. In period O nothing was given. The AA, glucose and oil were given in four divided doses over the day. N in urine and faeces was estimated by the Kjeldahl method. Pretreatment weight ranged from 110 to 215 kg. The effective weight loss per week over the whole period was 2·9 (Z), 3·0 (G_1), 2·5 (G_2) and 2·0 (M) kg. Faecal N loss was approximately 0·45 g/d, equalling the obligatory faecal N loss (FAO/WHO, 1973). Urinary N excretion decreased within 2 to 3 weeks to a constant low level, independent of the small amounts of amino acid, sugar and oil given. This level was individually different, but always higher than the obligatory urinary N loss reported by Scrimshaw (1972). N balance was negative in all periods. It improved with time due to the decrease in urinary N excretion, it became more negative when amino acids were omitted (O), but it was not influenced by the addition of glucose or oil to the amino acid (S, F).

Nitrogen balance (g/14 d)

				Subject			
					♂ 43 yr		
Period	L-amino acid 20 g	Glucose 20 g	Oil 9 g	♀51 yr Z	G_1	G_2	♀21 yr M
O				−113	−117		
A	+			−52	−61		
S	+	+		−36	−53		
F	+		+	−44	−49	−109	−77
S	+	+				−29	−37
O						−65	−61
A	+						−25
S	+	+					−22

Baird, I. M., Parsons, R. L. & Howard, A. N. (1974). *Metabolism.* **23**, 645.
FAO/WHO (1973). *Techn. Rep. Ser. Wld Hlth Org.* no. 522.
Scrimshaw, N. S., Hussein, M. A., Murray, E., Rand, W. M. & Young, V. R. (1972). *J. Nutr.* **102**, 1595.

The net exchange of amino acids from muscle of fed and starved sheep.

By D. B. LINDSAY and J. W. STEEL, *Department of Biochemistry, ARC, Institute of Animal Physiology, Babraham, Cambridge CB2 4AT,* and P. J. BUTTERY, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

The net exchange of amino acids by muscle in fed and starved sheep has been determined using the technique of Domanski *et al.* (1974). A–V differences for amino acids were determined using a site for sampling venous blood which is predominantly (>90%) drained from hind-limb muscle. Blood flow was determined by the ^3H_2O equilibration technique of Pappenheimer & Setchell (1972). Fed sheep were given 90% of their *ad lib.* intake of grass cubes in 24 equal hourly portions. Measurements were made during this feeding regime and at about 48, 72 and 95 h following feeding.

For most amino acids, the arterial concentration was lower in starved than in fed sheep. The decrease was significant, however, only for alanine, serine, valine, phenylalanine and tyrosine. Glycine and 3-methyl-histidine increased in concentration during starvation, although only for the latter was the increase significant.

There was considerable variation in output or uptake of amino acids in fed sheep, the only significant value being an output of phenylalanine. In starved sheep eight amino acids had an output significantly greater than zero:

	(μmol/kg muscle per min)	
	Fed (4)	Fasted (12)
Alanine	0·32±2·14	5·12±0·66
Serine	−1·32±1·36	1·03±0·15
Threonine	−0·97±0·51	2·51±0·67
Histidine	−1·24±0·58	0·60±0·22
Leucine	−1·72±1·50	1·34±0·35
Isoleucine	0·81±1·11	0·42±0·16
Valine	−3·20±3·65	2·04±0·61
Phenylalanine	1·00±0·33	0·53±0·23

(Values are means ± SEM. Figures in parentheses indicate nos. of experiments. Negative values (in fed sheep) indicate uptake).

The mean apparent output of glycine was almost as large as that of alanine, but there were large variations between animals so that the output was not significantly different from zero (4·75±2·32 μmol/min per kg).

If the muscle studied is representative of skeletal muscle and the total muscle mass is taken as 10 kg, the total α-amino-N output is about 4·4 g/d, equivalent to a breakdown of muscle protein of about 30 g/d. The rate of breakdown can also be calculated from the values for the output of individual essential amino acids and the corresponding amino acid content of the muscle. Leucine, isoleucine, phenylalanine, lysine, arginine and methionine gave estimates which agreed well with that obtained from the total amino-N output. Values more than twice as great were obtained when the outputs of threonine, histidine or valine were the basis of the calculation.

Domanski, A., Lindsay, D. B. & Setchell, B. P. (1974). *J. Physiol.* **242**, 30.
Pappenheimer, J. R. & Setchell, B. P. (1972). *J. Physiol.* **226**, 48.

The use of urinary ammonia as end-product in measurements of total protein turnover with ^{15}N. By J. C. WATERLOW, M. H. N. GOLDEN and MARY SUSAN ELL, *Clinical Nutrition and Metabolism Unit, London School of Hygiene and Tropical Medicine, and Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica*

In recent years the method most widely used for measuring total protein turnover in man is that described by Picou & Taylor-Roberts (1969), in which [^{15}N]glycine is infused and the turnover rate calculated from the labelling of urinary urea at plateau. In theory any end-product can be used, provided that it is derived from a single precursor pool, which is also taken to be the precursor pool for protein synthesis (Shipley & Clark, 1972).

A disadvantage of urea is the slow turnover rate of its pool, particularly in subjects on a low protein diet, so that in adults measurements may take as long as 3 d. We have therefore explored the use of urinary ammonia as end-product because it has a small pool turning over rapidly.

Two sets of results are presented: (a) in children given a constant infusion of [^{15}N]glycine, turnover rates calculated from NH_3 were two-thirds of those calculated from urea; (b) in obese adults given a single dose of [^{15}N]glycine, our estimate of the turnover rate from NH_3 excretion was 10% greater than that from urea. In both series the rank correlation between results with the two end-products was highly significant.

Picou, D. & Taylor-Roberts, T. (1969). *Clin. Sci.* **36**, 283.
Shipley, R. A. & Clark, R. E. (1972). *Tracer Methods for In Vivo Kinetics*. New York and London: Academic Press.

The relationship between the growth of the DNA-unit in muscle and protein turnover. By D. J. MILLWARD, P. BATES and G. LAURENT, *Clinical Nutrition and Metabolism Unit (Department of Human Nutrition, London School of Hygiene and Tropical Medicine), Hospital for Tropical Diseases, 4 St Pancras Way, London NW1 2PE*

The post-natal growth of individual muscles involves not only an increase in the number of nuclei but also in the amount of protein associated with each nucleus, i.e. the size of the DNA-unit. In the rat the protein to DNA ratio of some muscles increases fivefold. At the same time the fractional synthesis rate of protein (FSR) falls from 29 to 5% per day (Millward, Garlick, Stewart, Nnanyelugo & Waterlow, 1975). We are proposing a model in which protein synthesis and breakdown are defined in terms of amounts per nucleus and which describes the relationship between these amounts and the DNA-unit size. The model is based on two premises: (1) the amount of protein synthesis per nucleus cannot in practice be markedly increased; (2) the amount of protein breakdown per nucleus reflects DNA-unit size and the fractional breakdown rate (FBR), which in turn reflects the growth rate of the muscle as well as the muscle type.

The first premise is based on the following experimental results: in the rat throughout post-natal development the synthesis rate per unit DNA in a particular muscle does not markedly change. In different muscles in fully grown rats and fowl, synthesis rates per nucleus are the same even though DNA-unit sizes vary by several fold. When growth is accelerated during nutritional rehabilitation of rats or during work-induced muscle growth in the fowl, rates of synthesis per nucleus are increased, but only by about 50% over control values.

The second premise is based on the following observations of how the FBR varies in muscle. During development the FBR is related to the growth rate (Millward *et al.* 1976). When the growth rate is increased during work-induced or catch-up growth the FBR increases, and when growth is interrupted by dietary or hormonal manipulation the FBR falls (Millward, Garlick, Nnanyelugo and Waterlow, 1976). Finally, in adult rats and fowl, the FBR is higher in heart and red muscles than in white muscles.

The implications of the model will be discussed in terms of (a) the relationship between tissue function, the FBR, and the upper limit to cell size in muscle and other tissues, (b) the relationship between potential growth rate and DNA-unit size in muscle and (c) the importance of nuclear proliferation in enabling the further growth of mature muscles.

Millward, D. J., Garlick, P. J., Stewart, R. J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975). *Biochem. J.* **150**, 235.

Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976). *Biochem. J.* **150**, 185.

An interaction of leucine, isoleucine and valine in the diet of the growing pig. By S. J. TAYLOR, D. J. A. COLE and D. LEWIS, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

An interaction between leucine, isoleucine and valine has been established in the diet of the growing pig by the study of growth and of metabolic parameters.

A basal diet was formulated using barley, tapioca, blood meal, yeast protein and synthetic amino acids to be marginally adequate in isoleucine (0·38%). The diet contained 1·3% leucine, 0·66% valine and adequate levels of other amino acids. Four dietary treatments were developed by the addition of synthetic isoleucine and leucine: levels of 0·38% and 0·45% isoleucine were combined with 1·3% and 2·0% leucine in a factorial design. The diets were fed to 32 pigs (25–55 kg live weight), there being 8 replicates of the 4 treatments.

The effects of diets on growth rate, food utilization, carcass quality, plasma urea nitrogen and amino acids were recorded. A blood sample was taken from the jugular vein at 40 kg live weight for plasma studies and carcass quality was assessed by physical dissection of the ham joint at the end of the experimental period. The results were:

Treatment	Basal diet	Basal diet + isoleucine	Basal diet + leucine	Basal diet + isoleucine and leucine
% of air dry diet	0·38% isoleucine 1·3% leucine	0·45% isoleucine 1·3% leucine	0·38% isoleucine 2·0% leucine	0·45% isoleucine 2·0% leucine
Growth rate	628·5 (bc)	651·5 (ab)	606·1 (c)	658·7 (a)
FCE (kg feed/kg gain)	2·58 (a)	2·45 (b)	2·71 (c)	2·43 (b)
Lean in ham (%)	65·08 (a)	66·38 (a)	62·9 (b)	66·26 (a)
Plasma urea nitrogen (mg/100 ml)	9·26	8·05	9·41	9·64

Results followed by a common letter are not significantly different at the level 5%

The results indicate that isoleucine was marginally deficient in the basal diet and a slight improvement in performance resulted on raising the isoleucine level to 0·45%. However, an increase in dietary leucine from 1·3% to 2·0%, at the lower level of isoleucine, resulted in a reduction in growth performance. This was probably due to a further reduction in the isoleucine available to the animal as a result of the increase in leucine. This conclusion was supported by the improvement in performance which resulted at the higher dietary leucine level when there was also an increase in dietary isoleucine.

The plasma free valine and isoleucine concentrations were depressed when the dietary leucine level was increased. Such a response has been used as evidence to establish the interaction between leucine, isoleucine and valine in the pig (Oestemer *et al.* 1973; Mitchell *et al.* 1968; Henry *et al.* 1976). However, the interaction has not previously been shown to influence growth performance and other production parameters in the growing pig.

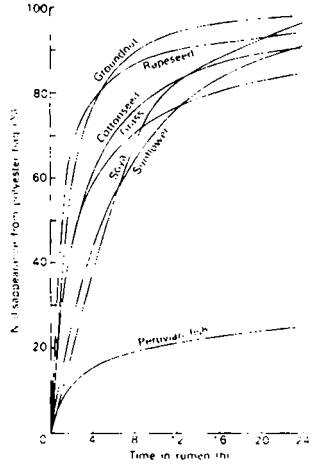
Mitchell, J. R., Becker, D. E., Harmon, B. G., Norton, H. W. & Jensen, A. H. (1968). *J. Anim. Sci.* **27**, 1327.
Oestemer, G. A., Hanson, L. E. & Meade, R. J. (1973). *J. Anim. Sci.* 36, 674.
Henry, Y., Duée, P. H. & Rerat, A. (1976). *J. Anim. Sci.* **42**, 357.

Rate and extent of protein degradation in the rumen. By J. C. MATHERS, C. M. HORTON and E. L. MILLER, *Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX*

Available data on the proportion of feed proteins degraded in the rumen have been obtained from measurement of protein passing to the abomasum or duodenum in animals fitted with cannulae postruminally, and subtraction of estimates of microbial and endogenous protein contributions. These techniques are difficult and time-consuming and provide only single estimates of the degradability of proteins when used in particular feeding regimens.

Bailey (1962) studied the disappearance of nitrogen and other components from feedstuffs incubated in polyester bags placed within the rumen and it has been suggested by E. R. Ørskov (personal communication) that such a simple, rapid technique could be used to estimate the extent of protein degradation in the rumen.

To test this hypothesis, two ruminally cannulated sheep were fed 840 g/d grass nuts. Sieved samples (Schoeman *et al.* 1972) of cottonseed, sunflower seed, groundnut, rapeseed and Peruvian fish meals were evaluated in sheep A, while cottonseed, dried grass and toasted soyabean meals were evaluated in sheep B. For each feedstuff, on each of 2 d, six tared polyester bags (20 cm×9 cm; 0·15 mm dia. pore) containing 4–5 g DM were suspended in the rumen and one bag was removed after 2, 4, 6, 8, 12 and 24 h. Then the bags were washed in cold tap water, dried to constant weight (100°) and the N content of the residual feedstuff was determined.



The results are shown in the figure where the curves are drawn from fitted hyperbolic functions (R=0·89–0·99). At short incubation times (4–6 h), the polyester bag technique gave estimates of N disappearance similar to estimates, for the same feed samples, of degradation in the rumen obtained from measurements of non-ammonia-N flow to the small intestine. DM disappearance from the bags at this time ranged from 15–80%.

We are indebted to A. Z. Mehrez for details of the polyester bag and advice on the procedure and to P. M. Lerman for computational assistance.

Bailey, C. B. (1962). *Can. J. Anim. Sci.* **42**, 49.
Schoeman, E. A., De Wet, P. J. & Burger, W. J. (1972). *Agroanimalia* **4**, 35.

Ammonia concentration and protein synthesis in the rumen. By A. U. OKORIE, P. J. BUTTERY and D. LEWIS, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

The concentration of ammonia in the rumen which promotes maximal synthesis of protein by rumen micro-organisms is one of the many factors which have to be considered when attempting to predict the effectiveness of including non-protein-nitrogen in ruminant diets.

Wethers (*c.* 45 kg) each fitted with a duodenal re-entrant cannula and a rumen fistula, were given 800 g daily of a low-protein diet using a continuous feeding apparatus. The diet: (g/kg) corn starch 310, glucose 200, straw 200, barley 100, ground grass 60, Nutramol (a molasses and peat mixture) (Rumenco Ltd, Burton-on-Trent) 50, vegetable oil 30, [vitamins and minerals] provided 5·2 g N/d. For three-week periods urea was continuously infused into the rumen. On days 18 to 21 $^{35}SO_4$ was added to the infusate and the total flow of microbial protein at the proximal duodenum was determined using the techniques described by Oldham & Ling (1976) and Beever *et al.* (1974).

Rumen NH_3 concentrations were monitored. After each three-week period the concentration of urea in the infusate was changed and the procedure repeated. Some of the results obtained are presented in Fig. 1 and indicates that maximal protein synthesis is achieved when the rumen NH_3 concentration reaches 5×10^{-3} mol/l; an observation consistent with the in vitro observations of Satter & Slyter (1974).

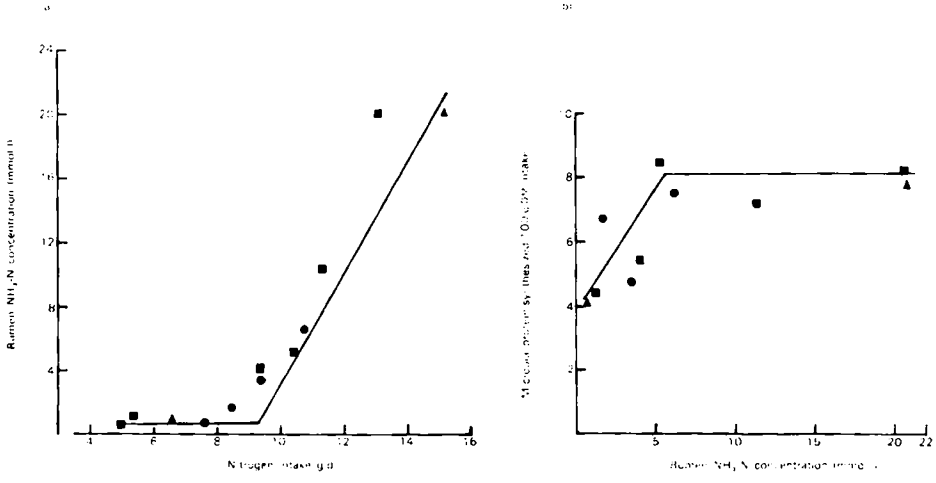


Fig. 1. (a) Effect of total N intake on rumen NH_3 concentration, (b) the effect of rumen NH_3 concentration on microbial protein passage at the proximal duodenum. (▲, ■, ●, indicate different sheep).

Satter, L. D. & Slyter, L. L. (1974). *Br. J. Nutr.* **32**, 199.
Oldham, J. D. & Ling, J. R. (1977). *Br. J. Nutr.* **37**, 333.
Beever, D. E., Harrison, D. G., Thomson, D. J., Cammell, S. B. & Osbourn, D. F. (1974). *Br. J. Nutr.* **32**, 99.

Health and performance of dairy cows fed high levels of urea for a complete lactation. By R. J. TREACHER, *Institute for Research on Animal Diseases, Compton, Newbury, Berks. RG16 0NN*

The effect of feeding up to 40% of the dietary nitrogen as urea has been investigated using two groups of eight British Friesian cows. A basic diet of straw, lucerne nuts and barley nuts was fed from five weeks before calving until the end of lactation. One group was given a production concentrate containing 2·9% urea and the other a concentrate containing fish meal and ground nut. The two concentrates were isoenergetic and isonitrogenous and both contained 6·5% dehydrated lucerne (Conrad & Hibbs, 1966). Cows were fed according to ARC (1965) recommendations for energy and protein, and consumed up to 10·9 kg of the urea concentrate daily, an intake of 316 g urea. Performance results over the first 10 weeks of lactation were:

	Urea-fed group (8)	Control group (8)
Total feed intake (kg):		
Barley straw	315	315
Barley nuts	266	253
Concentrates	532	490
Lucerne nuts	98	89
Milk production:		
Peak yield (kg/d)	28·0	29·7
Total yield (kg)	1703	1810
Fat (%)	4·08	3·79
Protein (%)	3·01	2·86
Lactose (%)	4·78	4·85
Live weight loss (% post-calving weight)	5·4	7·4
Calving to first oestrus (d)	48	38
Calving to conception (d)	126 (6 cows)	112 (5 cows)

Milk yield declined earlier in the urea-fed group, but rates of decline were similar in the two groups (1·75%/week).

Blood ammonia concentrations were similar in the urea-fed group and the control group. In the first four cows which have completed a lactation the plasma activities of glutamate dehydrogenase, ornithine carbamyl transferase, and sorbitol dehydrogenase were similar in the urea-fed group and the control group for 12 weeks post-calving and thereafter were lower in the urea-fed group. Otherwise there were no differences in blood composition between groups for any week.

There is no evidence that the urea ration raised plasma ammonia levels, gave rise to liver damage, depressed feed intakes, significantly impaired fertility, or produced mineral deficiencies. The depression in milk yield and the absence of anaemia with the urea ration is in sharp contrast with the results of experiments reported by Treacher *et al.* (1976) in which a reduction of protein in a ration not containing urea to 25% below ARC standards, produced no significant decrease in milk yields of dairy cows, but blood haemoglobin levels and red blood cell concentrations were decreased.

ARC (1965). *The nutrient requirements of livestock, No. 2 Ruminants.* London: Her Majesty's Stationery Office.

Conrad, H. R. & Hibbs, J. W. (1966). Dairy Sci. Series Dept., Ohio Agric. Res and Dev. Centre, 10 (4), Wooster, Ohio.

Treacher, R. J., Little, W., Collis, K. A. & Stark, A. J. (1976). *J. Dairy Res.* **43**, 357.

Hepatic gluconeogenesis and fat metabolism in fed and fasted lactating dairy cows in vivo. By G. D. BAIRD, I. M. REID, M. A. LOMAX, H. W. SYMONDS, C. J. ROBERTS and D. MATHER, *ARC, Institute for Research on Animal Diseases, Compton, Berks. RG16 0NN*

The catheterization technique of Baird *et al.* (1975) has been used to determine hepatic metabolite production rates in two mature British Friesian lactating dairy cows in vivo. In the experiment the cows were fed to requirement initially, then subjected to 6 d of fasting. The values give in the table, except for those for free fatty acids (FFA), are for production rates obtained (a) in the fed state immediately before fasting and (b) after 4 d (96 h) of fasting. The values for FFA are the average of observations made on 3 separate days of feeding and on 4 separate days of fasting.

	Production rate			Production rate	
Metabolite	Fed	Fasted	Metabolite	Fed	Fasted
Gluconeogenesis			Fat metabolism		
Glucose (mmol/min)	+5·5	+1·6	Acetate (mmol/min)	+4·6	+0·4
Propionate (mmol/min)	−3·6	0	Hydroxybutyrate (mmol/min)	+1·9	+3·4
Lactate (mmol/min)	−2·5	−1·8	Acetoacetate (mmol/min)	−0·7	+0·6
Pyruvate (mmol/min)	−0·2	−0·2	Butyrate (mmol/min)	−1·5	0
Glycerol (mmol/min)	−0·2	−0·5	FFA (mg/min)	−105	−317

(Values were calculated as described by Katz & Bergman (1969). Blood metabolite concentrations assayed enzymically, except that of FFA, which was determined by thin-layer chromatography, and of propionate and butyrate by gas liquid chromatography.)

The results indicate that fasting caused a 71% decrease in hepatic glucose production. In the fed state propionate, (lactate+pyruvate) and glycerol could contribute maximally 33%, 25% and 2% respectively to hepatic glucose production. These figures are not dissimilar to those obtained earlier by Bergman and co-workers in fed sheep (Bergman, 1973). In the fasted state the corresponding figures are 0%, 63% and 16% respectively. The increased potential importance of lactate and glycerol for gluconeogenesis in fasting is thus emphasized.

Fasting caused a major decrease in apparent hepatic acetogenesis and a threefold increase in net hepatic ketogenesis. The observed uptake of butyrate and FFA was in fact only sufficient to account for some 68% of total ketone body and acetate production by the liver in the fed state. During fasting, butyrate uptake by the liver ceased, while free fatty acid uptake trebled. Even so, the free fatty acid only accounted for some 73% of the hepatic ketogenesis and acetogenesis in this instance too.

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Pathogenesis of fatty liver in fasted cows. By I. M. REID, R. A. COLLINS, C. J. ROBERTS, H. W. SYMONDS and G. D. BAIRD, *ARC Institute for Research on Animal Diseases, Compton, Newbury, Berks. RG16 oNN*

Cows deprived of food for 6 d develop a fatty liver. On the basis of ultrastructural (Reid, 1973) and chemical studies (Brumby *et al.* 1975), we suggested that increased hepatic uptake of free fatty acids (FFA) and decreased hepatic production of triglyceride (TG) were possible causes of the fatty liver. The present study was designed to test this hypothesis by using cows with cannulae implanted in the portal and hepatic veins.

Two British Friesian lactating cows were surgically prepared as described by Baird, Symonds & Ash (1975). Both cows were fasted for 6 d and blood samples were collected from the carotid artery, portal vein and hepatic vein cannulae on days 1, 2, 4 and 6 of the experimental period and on 2 control days before the start of the fasting period when both cows were fed normally. Blood lipid concentrations were determined on extracts of plasma (Atkinson *et al.* 1972) by a TLC-charring method (Kritchevsky *et al.* 1973). The production rates of lipid fractions were calculated from concentrations in arterial, portal and hepatic-venous blood, and from the rates of blood flow in the portal and hepatic veins (Baird *et al.* 1975).

The results are given in the table:

	Production rate* (mg/min)			
	FFA		TG	
Day	Cow 1	Cow 2	Cow 1	Cow 2
Fed				
1	− 61	− 61	+658	+271
2	−241	− 69	+ 99	+313
Fasted				
1	−329	−285	−196	+ 59
2	− 11	−277	+ 43	− 10
4	−264	−133	− 23	−121
6	−731	ND	−216	ND

*A positive value indicated net production by the liver and a negative value net utilization by the liver.
ND, not determined.

They showed that hepatic uptake of FFA doubled, on average, in cow 1 and tripled in cow 2 during fasting. In the fed state there was a net hepatic production of TG in both cows which decreased and became a net hepatic uptake during fasting.

The results, while only from two cows, provide direct evidence that increased hepatic uptake of FFA and decreased hepatic output of TG are implicated in the fatty liver of fasted cows.

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Lipogenesis in genetically obese rats (fa/fa). By D. A. YORK and V. GODBOLE, *Department of Physiology and Biochemistry, The University, Southampton SO9 3TU*

Both the liver and adipose tissue are major sites of fatty acid synthesis. However, it is not known whether the relative contribution of these two tissues to the total body lipogenesis changes in the obese fat.

In situ lipogenesis in liver and adipose tissue (4 sites) was measured after i.v. injection of ^3H_2O (Lowenstein, 1971). The results, summarized in the table show that the rate of fatty acid synthesis in the obese rat in both the liver and adipose tissue was tenfold greater than in the tissues of lean rats at 6 weeks of age. In lean rats the liver was the main site of fatty acid synthesis. In obese rats the contribution of liver and adipose tissue to total fatty acid synthesis was more evenly divided, mainly because of the increased mass of adipose tissue. The rate of hepatic lipogenesis increased with age in both the lean and obese rats, whereas the rate of lipogenesis in adipose tissue did not change in lean rats but decreased in obese rats. Thus although the enhanced synthesis of fatty acids in 6 week old obese rats resulted from an increase in synthesis in both liver and adipose tissue, the further increase with age was entirely due to an increase in hepatic lipogenesis. The liver is thus of major importance in the hyperlipogenesis of 'fatty' rats.

			Fatty acid synthesized (μmoles)		
Rat	Age (weeks)	Site	(μmoles/g per h)	(μmoles/tissue per h)	Liver / Adipose tissue
Lean	6	Liver	0·27±0·05	1·27± 0·44	
		Adipose A	0·14±0·01	0·08± 0·01	7·42
		Adipose B	0·07±0·01	0·09± 0·02	
fa/fa	6	Liver	2·65±0·74	22·09± 5·76	
		Adipose A	1·06±0·11	4·90± 0·54	1·95
		Adipose B	0·76±0·04	6·41± 0·25	
Lean	13	Liver	0·48±0·15	3·98± 1·19	
		Adipose A	0·08±0·04	0·32± 0·17	5·94
		Adipose B	0·11±0·03	0·35± 0·08	
fa/fa	13	Liver	4·70±1·06	61·80±10·01	
		Adipose A	0·21±0·02	2·40± 0·18	10·77
		Adipose B	0·10±0·01	3·27± 0·55	

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Serum cholesterol and triglycerides concentrations in vegans. By T. A. B. SANDERS and F. R. ELLIS, *Department of Pathology, Kingston Hospital, Kingston, Surrey* and J. W. T. DICKERSON, *Department of Biochemistry, University of Surrey, Guildford*

We have estimated serum cholesterol and triglycerides concentrations in a series of vegan and omnivore subjects. Vegan subjects were contacted through the Vegan Society and omnivore subjects were healthy hospital staff, their relatives and friends. Blood samples were obtained from the subjects by venepuncture after an overnight fast and serum cholesterol and serum triglycerides were determined by routine automated chemical methods. The results of the determinations in 22 vegans and 22 omnivores were:

(Mean values in mmol/l)

	Serum cholesterol	Serum triglycerides
Vegans	4·1 (3·0–5·4)	0·95 (0·60–1·90)
Omnivores	6·1 (3·9–7·7)	1·35 (0·45–2·95)

The concentrations of both serum cholesterol and serum triglycerides were significantly lower in the vegans than in the omnivores ($P<0·01$).

A vegan diet comprised of unrefined cereals, nuts, pulses, fruit and vegetable products appears to be adequate providing it is supplemented with vitamin B_{12} (Ellis & Mumford, 1967; Ellis & Montegriffo, 1970). The typical vegan diet tends to contain no animal protein, no cholesterol, a low proportion of saturated fatty acids and high proportions of polyunsaturated fatty acids, fibre, plant sterols and unrefined carbohydrates; all factors that apparently lower serum lipid concentrations. It is suggested that a vegan diet may be the diet of choice for the treatment of certain types of hyperlipidaemia.

We are grateful to Dr B. W. Meade for the biochemical estimations and acknowledge support from a grant from the South West Thames Regional Health Authority De-centralised Research Fund to F.R.E. and J.W.T.D.

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Viscosity and the action of unavailable carbohydrate in reducing the post prandial glucose and insulin levels. By D. J. A. JENKINS, A. R. LEEDS and M. A. GASSULL, *MRC Gastroenterology Unit, Central Middlesex Hospital, London NW10 7NS* and D. V. GOFF and T. M. S. WOLEVER, *University Laboratory of Physiology, Oxford* and K. G. M. M. ALBERTI, *University of Chemical Pathology and Human Metabolism Unit, General Hospital, Southampton*

The unabsorbable carbohydrates guar and pectin have been shown to reduce the post prandial rise in glucose and insulin levels in both normal and diabetic subjects. Both substances increase the viscosity of aqueous solutions. In order to see whether this property was related to the action of these substances, guar was partially hydrolysed so that it no longer increased the viscosity of aqueous solutions. A test meal containing 50 g glucose was made up in 400 ml water and given to 4 male subjects in the morning, after an overnight fast. Blood samples were obtained for glucose and insulin analysis at the times shown in the table:

Time (min)	0	15	30	45	60	90	120
	(Mean±SEM blood glucose mmol/l)						
Control	4·9±0·4	6·8±0·6	8·1±0·9$^{\bullet}$	8·4±1·6$^{\bullet}$	6·8±1·3	5·6±0·7	4·4±0·5$^{\bullet\bullet}$
Hydrolysed guar	4·7±0·3	6·2±0·6	8·7±0·7$^{\bullet\bullet\bullet}$	7·7±0·8	6·3±0·6	4·2±0·6	4·1±0·5$^{\bullet}$
Unhydrolysed guar	4·9±0·4	5·3±0·4	6·4±0·8	6·4±1·1	6·4±1·2	5·9±0·8	5·7±0·6
	(Mean±SEM serum insulin mU/l)						
Control	4±1	19±4$^{\bullet}$	44±6	47±4$^{\bullet\bullet}$	34± 7$^{\bullet}$	27±4	5±1
Hydrolysed guar	5±1	24±3	52±9$^{\bullet}$	53±9$^{\bullet}$	49±10$^{\bullet}$	16±6	12±5
Unhydrolysed guar	5±1	12±3	24±5	22±3	17±3	16±5	15±3

Significance difference from unhydrolysed guar: $^{\bullet}P<0.05$, $^{\bullet\bullet}P<0.02$, $^{\bullet\bullet\bullet}P<0.01$.

On two other separate occasions 14·5 g hydrolysed or 14·5 g unhydrolysed guar was added to the liquid meal and the experimental procedure repeated. The results shown in the table indicate that in the control situation and when hydrolysed guar was given there was little difference in the insulin or glucose levels, but between 30 and 45 min the mean glucose concentration in both the control situation and when hydrolysed guar was given was higher than when the unhydrolysed guar was included in the test meal. This pattern was reversed at 120 min. The mean insulin values for both the control and hydrolysed guar experiments were also higher than those obtained when unhydrolysed guar was given over the 15 to 60 min period.

It is suggested that the action of guar and also of other unabsorbable carbohydrates in reducing the post prandial rise in glucose and insulin might be related to their ability to form viscous solutions and so perhaps slow gastric emptying or diffusion across the unstirred layer in the small intestine.

Blood glucose profiles in man after ingestion of different loadings of glucose and glucose/fructose mixtures. By M. W. KEARSLEY and G. G. BIRCH (Introduced by I. MACDONALD), *National College of Food Technology, University of Reading, St George's Avenue, Weybridge, Surrey*

When starch is almost completely hydrolysed, up to 50% of the glucose formed can be enzymically converted to fructose. These isomerized glucose syrups are finding progressively wider application as natural sweeteners in the food and drink industries. Consequently it was decided to investigate the physiological response to the ingestion of isomerized glucose syrup and different test carbohydrate loadings as judged by the glucose tolerance test.

On six separate occasions after an overnight fast, six apparently healthy adult volunteers (four male and two female) ingested one of six test carbohydrates: 50 g, 25 g and 10 g glucose, 20 g and 10 g isomerized glucose syrup and 50 g fructose. The order of ingestion was randomized using a 6×6 Latin Square design. Capillary blood samples were taken at 30 min intervals after ingestion for a total period of 2 h. Blood glucose concentration was determined using the GOD-Perid Method (Werner *et al.* 1970).

No significant difference was observed between the maximum blood glucose concentrations induced by 50 g and 25 g glucose. However, a significant difference ($P \leqslant 0{\cdot}02$) was found in the response to 50 g and 10 g glucose. These findings are consistent with those of Jourdan (1972) and a possible explanation of the effect is that when the quantity of glucose ingested is sufficiently high, the maximum rate of stomach emptying is a limiting factor in the rate of entry of glucose into the blood stream.

Blood glucose response to the ingestion of fructose alone produced variable elevations as previously reported in the literature (Cornblath *et al.* 1963; Matschinsky *et al.* 1972). At the lower levels of challenge, the response to 20 g isomerized glucose syrup (containing 10 g glucose) was significantly higher ($P \leqslant 0{\cdot}05$) than that to 10 g glucose, possibly as a result of a high conversion of fructose to glucose at this level.

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The health of vegans during pregnancy. By J. THOMAS and F. R. ELLIS, *Department of Pathology, Kingston Hospital, Kingston, Surrey,* and P. L. C. DIGGORY, *Department of Obstetrics and Gynaecology, Kingston Hospital, Kingston-on-Thames, Surrey*

A number of recent papers (Hardinge & Stare, 1954; Wokes, Badenoch & Sinclair, 1955; Smith, 1962; West & Ellis, 1966; Ellis & Montegriffo, 1970; Ellis, West & Sanders, 1975) have surveyed the general health of vegans whose diet consists entirely of vegetable matter, i.e. no meat, fish, or dairy products, and there are a few individual case reports none of which include any pregnant women. In view of the current discussion over world food shortage and the increasing use of vegetable products to replace animal matter in the diet, it is important to know whether a vegan diet has any adverse effect either on the health of mothers during and after pregnancy or on the health of their babies.

Information was obtained from fourteen vegans (28 pregnancies) and eighteen controls (41 pregnancies) who were drawn from healthy laboratory and office personnel and the routine post-natal clinic.

The average age of the vegans at the time of their first pregnancy was 30 years (range 24–39) and of the controls 25·5 years (17–36). The findings are summarized in the table:

	Vegans	Controls
Live births	24 (86%)	36 (88%)
Still births/miscarriages	4 (14%)	5 (12%)
Toxaemia of Pregnancy	4 (17%)	7 (19·5%)
Anaemia of Pregnancy	3 (12·5%)	2 (5·5%)
Iron Supplements	3 (21%)	12 (66%)
Breast fed, 3 months +	23 (96%)	12 (31%)
Birth weight (kg)	3·1±0·8	3·3±1·2

It can be seen from the table that still births, toxaemia and anaemia of pregnancy were not significantly more prevalent in vegans than in controls, in spite of the fact that only 3% of vegans took iron supplements. None took vitamin B_{12} supplements. The difference in the birth weights of the two groups is not statistically significant. There was only one case of congenital abnormality (congenital dislocation of the hip) in each group. There was no significant difference in the pre- and post-natal health of the mothers in either group.

The vegan mothers showed an overwhelming desire to breast feed their babies, possibly to ensure that they too have a vegan diet. The babies were also weaned onto a vegetable preparation instead of cow's milk in the vegan group.

This study shows that vegan women undergo pregnancy whilst continuing their vegan diet without any adverse effect on themselves or their offspring, the only difference being the increased incidence of breast feeding which is a positive advantage to the child.

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Feeding practices and food intake of children under two years of age.

By JANE MORGAN and PAMELA MUMFORD, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

An investigation in December 1973–January 1974 into feeding practices of 707 children aged 0–24 months representing a national cross-section of the British population provided 584 reliable quantitative 7-d food intake records, and information on social class, family size and location. 126 subjects were followed up in June, 1975 (Morgan *et al.* 1976). 288 (63%) of the remainder satisfactorily completed a questionnaire to give more detailed data on feeding practices. The proportion of respondents from each of the social gradings (AB 12%; C1 25%; C2 49%; DE 14%) remained identical throughout.

12% of the children were wholly breast fed, and 58% bottle fed. The duration of bottle feeding was not affected by social class. However, those wholly breast feeding continued on average 2 months longer in class AB than in class DE, and where mixed breast and bottle feeding was adopted, the breast feeding continued 1 month longer in class AB than C2 and DE.

Daily energy intakes were not substantially different from Recommended Intakes (RDI) (DHSS, 1969):

		Energy (MJ)			Protein (g)		
			Sample			Sample	
Months	(n)	RDI*	Mean	Range	Minimum* requirement	Mean	Range
0–3	(40)	2·3	2·5	1·3–5·0	13	24·9	11·2–45·9
3–6	(66)	3·2	3·4	1·6–5·6	14	30·7	10·3–57·6
6–9	(63)	3·8	3·7	2·2–5·7	15	32·9	17·0–56·7
9–12	(94)	4·2	4·0	2·3–7·1	16	35·6	19·8–59·6
12–24	(287)	5·0	4·9	2·0–7·4	19	41·3	15·5–67·0

*DHSS (1969)

However, in the 0–3 month age cohort (excluding breast-fed babies) 60% of children had intakes above the recommended level. From 6 months this trend was reversed, with 60% of the intakes below the recommended level. At age 0–3 months protein intake averaged 24 g/d (192% of minimum requirement), and many children had excessively high intakes. In all age cohorts there was at least a twofold range of intakes for both energy and protein; some 3 month old infants were eating as much as some toddlers. Food intake and health records of children at the upper and lower ranges for energy and protein gave no reason for their exclusion. However, some of the more extreme intakes of certain nutrients (particularly calcium, iron, sodium, energy and protein) were explicable by unusual feeding patterns. Boys consistently ate more than girls. There were no significant differences in energy and protein intakes by social class.

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Dietary habits and the effect of bedtime food drinks on sleep. By KIRSTINE ADAM, *University Department of Psychiatry, Morningside Park, Edinburgh EH10 5HF*

This study investigated the relationship between the usual later evening eating pattern and the effect on sleep of nourishing bedtime drinks. The sixteen subjects (10 women, 6 men) aged 52–67 (mean 59 years) were instructed to take no nourishment after 19.00 hours except their treatments at 22.00 hours: (A) placebo capsules, no energy value; (B) 'Flavoured Drink' specially formulated from soya, egg and sucrose, to match treatment D in composition (1260 kJ); (C) 280 ml hot milk (800 kJ); (D) Horlicks, a milk and cereal food in hot milk (1260 kJ). The experimental design was: Weeks 1 and 2, at home on treatment; Week 3, 1 adaptation+5 nights recorded at sleep laboratory on treatment (sleep recorded electrophysiologically); Week 4, at home, no treatment.

The above was repeated for each of the treatments A, B, C, D according to a Latin square.

Subjects energy intake through the day was calculated from diet diaries. For each subject a rating of 'predicted' benefit from later evening nourishment was made by: (energy intake after 21.00 hours/total energy intake after 17.00 hours) × 100 = N%.

These N% scores were ranked 1–16 in the order of increasing 'predicted' benefit from later evening nutrition, on the assumption that a bedtime food drink would most benefit the sleep of those who normally took most food later in the evening.

From each subject's recordings of sleep the mean minutes spent asleep on nights after capsules was subtracted from the mean after each of the three bedtime drinks. These differences in 'measured' benefit of later evening nourishment were ranked 1–16. The same procedure was used to rank the difference in the amount of wakefulness.

Spearman's rank correlation test was used to compare the 'predicted' rank order with the 'measured' order of benefit for each of the three bedtime drinks:

(Spearman's Rank Correlations df=14 (n-2) 2-tailed)

	Flavoured drink	Milk	Horlicks
Total time asleep	r_s 0·19 (NS)	r_s 0·64 $P<0·01$	r_s 0·51 $P<0·05$
Total wakefulness after first falling asleep	r_s 0·09 (NS)	r_s 0·51 $P<0·05$	r_s 0·55 $P<0·05$

NS, not significant.

Milk and Horlicks both gave significant positive correlations, i.e. the subjects who normally had a bedtime snack slept better after these food drinks than after capsules, whereas those who usually had little or nothing slept better after capsules. The lack of any correlation for the flavoured drink indicates that not just energy is important for the effects of later evening nutrition. There was also less wakefulness interrupting the first 6 h of sleep after Horlicks when compared with either of the other two bedtime drinks ($P<0·05$).