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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Thirteenth Meeting of the Nutrition Society was held at the Clinical Research Centre, Harrow, Middlesex HA1 3UJ, on Monday, 27 *February,* 1978, *when the following papers were read:*

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Protein synthesis in rat small intestine. By M. A. MCNURLAN, P. J. GARLICK and A. M. TOMKINS, *Department of Human Nutrition, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 1HT*

The rate of protein synthesis in the jejunal mucosa of the rat was estimated by two methods. The first method, constant infusion of [^{14}C]tyrosine over 6 h (Garlick *et al.* 1973), poses several problems. Because the specific activity of protein tyrosine is nearly 50% that of free tyrosine after 6 h, protein breakdown results in a significant loss of labelled protein and a continuing rise in the specific activity of the tissue free amino acid. Hence two essential conditions of this method are not met. Uncertainty also arises out of the large difference between the specific activity of free tyrosine in the plasma and in the tissue whenever tracer doses of labelled amino acid are given. As the table shows, results calculated from tissue free tyrosine ($k_{s,i}$) are 4-fold higher than those calculated using plasma ($k_{s,p}$) tyrosine specific activity.

In the second method a large dose of [^{14}C]leucine (100 μmol/100 g rat) was injected and incorporation into protein measured between 2 and 10 min after injection. This method is similar to that used by Henshaw *et al.* (1971) to measure protein synthesis in the liver. The large dose is intended to flood the animal with labelled leucine so that the specific activity in all compartments is similar and remains reasonably constant for some time after injection. During this short period the breakdown of labelled protein is negligible. Decisions about whether the plasma or the intracellular pool is the precursor for protein synthesis become less important since the rate of synthesis calculated from the specific activity of plasma leucine ($k_{s,p}$) is 70% of the rate calculated from tissue free leucine ($k_{s,i}$).

Estimates of the fractional rates of synthesis, k_s *(%/d±*SD)

	Constant infusion, [^{14}C]tyrosine		Single injection, 100 μmol [^{14}C]leucine	
	$k_{s,p}$	$k_{s,i}$	$k_{s,p}$	$k_{s,i}$
Liver	21± 1	59± 5	71±11	79±12
Jejunal mucosa	50±17	218±92	100±11	145±28

Comparison of the rates of synthesis obtained by the two methods of isotope administration for liver are in reasonable agreement. The rate of synthesis measured by the large dose is expected to be greater than that from constant infusion because it includes plasma protein synthesis.

The results show that protein synthesis in the jejunal mucosa is considerably faster than liver. Moreover, problems encountered in the measurement of protein synthesis in the gut mucosa are minimized by injection of a large dose of [^{14}C]leucine.

Garlick, P. J., Millward, D. J. & James, W. P. T. (1973). *Biochem. J.* **136**, 935.
Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971). *J. Biol. Chem.* **246**, 436.

Synthesis of albumin and of total plasma proteins by liver of fed and starved rats. By V. M. PAIN, P. J. GARLICK and M. A. MCNURLAN, *Department of Human Nutrition, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT*

Starvation has been reported to reduce the rate of synthesis of plasma albumin in the rat, both in absolute terms (Rothschild *et al.* 1968) and as a proportion of the total protein synthesis by the liver (Peters & Peters, 1972). In contrast, earlier workers (Wilson *et al.* 1967) claimed that albumin synthesis was particularly resistant to impairment by fasting. We have used two methods to investigate this problem. The first method was designed to identify selective effects on synthesis of albumin relative to total protein synthesis by the liver. Rats were given a large dose of [^{3}H]leucine (McNurlan *et al.* 1978) and killed 10 min later. Incorporation of radioactivity into immunoprecipitable albumin in the liver was measured relative to incorporation into total hepatic protein. In the second method, rates of synthesis of total plasma proteins and liver tissue proteins were measured by constant infusion of a tracer dose of [^{14}C]tyrosine for 6 h (Garlick *et al.* 1973). Synthesis of albumin relative to total plasma protein was estimated by immunoprecipitation from the plasma.

As shown previously (Garlick *et al.* 1975), starvation did not result in a change in the fractional rate of synthesis of liver tissue protein (% of liver protein/d), i.e. the absolute rate (g/d) declined in proportion to the protein content of the liver. The fractional rate of synthesis of total plasma proteins (% of intravascular pool/d) fell by 26%. Expressed as an absolute rate this is also in line with the decrease in liver size. The results in Table 1 show that in starvation there is no change in the distribution of protein synthetic activity in the liver between production of plasma proteins (or albumin alone) and that of hepatic tissue proteins.

Table 1. *Effect of starvation on synthesis of plasma proteins and of albumin as a percentage of total protein synthesis by the liver*

	Single dose of [^{3}H]leucine	Constant infusion of [^{14}C]tyrosine	
	Albumin	Albumin	Total plasma proteins
Fed	13·1	15·4	38·2
Starved: 24 h	11·3	—	—
48 h	13·4	15·2	34·4

Garlick, P. J., Millward, D. J. & James, W. P. T. (1973). *Biochem. J.* **136**, 935.
Garlick, P. J., Millward, D. J., James, W. P. T. & Waterlow, J. C. (1975). *Biochim. biophys. Acta* **414**, 71.
McNurlan, M. A., Garlick, P. J. & Tomkins, A. M. (1978). *Proc. Nutr. Soc.* **37**, xx.
Peters, T. & Peters, J. C. (1972). *J. biol. Chem.* **247**, 3858.
Rothschild, M. A., Oratz, M., Mongelli, J. & Schreiber, S. S. (1968). *J. clin. Invest.* **47**, 2591.
Wilson, S. H., Hill, H. Z. & Hoagland, M. (1967). *Biochem. J.* **103**, 567.

The effect of aging on protein turnover in the rat. By D. J. MILLWARD and P. C. BATES, *Department of Human Nutrition, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT*

During postnatal development the rate of protein turnover in rat skeletal muscle falls towards a particular maintenance rate characteristic of the individual muscle, this value being reached at about 3 months of age (Millward *et al.* 1978). We have now investigated the extent to which the maintenance rate of turnover changes with aging.

Rates of protein synthesis in the combined gastrocnemius and quadriceps muscles, heart and liver were determined by the constant infusion method with [^{14}C]tyrosine (Garlick *et al.* 1973) in a slow-growing hooded strain at 1 and 2 years of age. Studies of longevity of this strain in our department show that at 2 years these rats are well into the steep part of their mortality curve (Wheeler, personal communication).

In both the skeletal and cardiac muscles the turnover rate at 2 years was reduced to two-thirds of the rate at 1 year. Measurements showed that both the contractile and sarcoplasmic proteins were involved in these changes and it appeared that the reduced rate of protein synthesis reflected a fall in tissue RNA concentration rather than a change in RNA activity. There was no evidence for any change in liver since the synthesis rate at 2 years was within the range observed by us in much younger rats. These results will be discussed in terms of possible consequences or reductions in muscle protein turnover on muscle function.

Effect of aging on rates of protein synthesis

(All values are means and standard deviations of 4–6 determinations)

		Muscle		Protein synthesis (%/d)	
Age (d)	Weight (g)	Protein synthesis (%/d)	RNA activity (g protein synthesis/d per g RNA)	Heart	Liver
360	413±20	3·6±0·4	8·9±0·9	12·5±1·5	—
740	398±30	2·4±0·4	8·2±1·2	8·5±0·8	58±6

Millward, D. J., Bates, P., Laurent, G. J. & Lo, C. C. (1978). In *Protein Turnover and Lysosomal Function* [H. Segal and D. Doyle, editors]. London and New York: Academic Press.

Garlick, P. J., Millward, D. J. & James, W. P. T. (1973). *Biochem. J.* **136**, 935.

A perfused ruminant muscle preparation. By B. J. COWARD and P. J. BUTTERY, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

The usual approach to studying muscle metabolism in ruminants involves in vivo cannulation of the blood supply to a muscle mass (Lindsay *et al.* 1976). We have adopted an alternative approach and have developed a perfusion system for the sheep diaphragm.

A sheep was exsanguinated and the diaphragm excised. The phrenic vein draining the left crus was cannulated and the hemidiaphragm flushed with 5–10 ml of oxygenated, heparinized (200 IU/ml) Krebs-Henseleit bicarbonate buffer (39°). The dorsal and ventral edges of the hemidiaphragm were cut to allow blood to flow from the preparation. The diaphragm was rapidly transferred to the laboratory (approximately 5 min after exsanguination of the animal) and perfused for 180 min at 39°, using apparatus based on that designed for the rat hind limb (Ruderman *et al.* 1971). Routinely, the medium consisted of 150 ml Krebs-Henseleit bicarbonate buffer (pH 7·4) containing washed sheep erythrocytes (to give a final haematocrit of 10–15%), bovine plasma albumin, fraction V, (80 g/ml), penicillin (100 IU/ml), streptomycin (100 μg/ml), acetate (2·81 mmol/l), glucose (4·64 mmol/l), DL-β-hydroxybutyrate (1·32 mmol/l), propionate (1·16 mmol/l) and butyrate (0·85 mmol/l). The right half of the diaphragm was clamped off along the join of the two crura. The flow rate through the preparation was routinely 7–8 ml/min at a constant pressure of 70 mm Hg.

Many criteria of adequacy of perfusion have been investigated. Perfusion with dye clearly demonstrated the completeness of perfusion, an observation confirmed with latex, and perfusion with [^{3}H]insulin and 2-[^{14}C]aminoisobutyric acid. The water content of the muscle after perfusion was 77·3±0·73% (12) compared with 75·0±0·12% (6) for fresh muscle.

ATP:ADP after perfusion was 3·83±0·20 (3) compared with 2·76±0·12 (3) for fresh muscle. Efflux of K^+ from the muscle during perfusion for 180 min was 0·57±0·08 (5) mmol/30 g muscle. Lactate production by the muscle during 180 min perfusion was 1·30±0·11 (7) mmol/30 g muscle and pyruvate production was 16·4±1·5 (6) μmol/30 g muscle.

Large quantities of alanine were released into the medium during perfusion (84·73±10·32 μmol/30 g muscle per 180 min). While the ratio of alanine to tyrosine in fresh muscle was 3·14±0·04 (4), the ratio of these amino acids in the perfusate at the end of perfusion was 13·96±1·78 (4). The muscle utilized acetate, glucose, propionate and butyrate but released β-hydroxybutyrate.

As with most perfusion systems some deterioration does occur, however, the above system is a useful tool to study ruminant muscle metabolism.

B. J. Coward acknowledges receipt of the Keith and Dorothy Mackay Travelling Scholarship (University of New England, Australia).

Lindsay, D. B., Steel, J. W. & Buttery, P. J. (1976). *Proc. Nutr. Soc.* **36**, 33A.
Ruderman, N. B., Houghton, C. R. S. & Hems, R. (1971). *Biochem. J.* **124**, 639.

Protein turnover and whole body nitrogen metabolism in the growing pig.

By B. K. EDMUNDS and P. J. BUTTERY, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD* and C. FISHER, *Unilever Research Ltd, Colworth House, Sharnbrook, Bedford, Beds.*

We present some preliminary results combining protein turnover rates, nitrogen balance and carcass analysis data in the young pig.

Turnover rates were measured in various organs and tissues by constant infusion of L-[4,5(*n*)-^{3}H]lysine for 10 h into pigs (about 25 kg) via ear vein catheters (Waterlow & Stephen, 1967). Specific activities of [^{3}H]lysine in plasma and tissue samples were determined.

N balance and carcass analyses were obtained from six gilts (starting weight 19·5±0·24 kg), fitted with bladder catheters and given a normal 'grower' diet. Balance results were obtained over four collection periods, each of 4 d duration. Animals were then slaughtered, fresh tissue weights recorded and their crude protein (N×6·25) content determined. The results are presented in the table.

Tissue	Fractional synthetic rate/d•	Protein content (g)†	Protein synthesized (g/d)
Liver	0·369±0·10	113·9± 4·3	42·0
Kidney	0·120±0·03	19·7± 1·2	2·4
Heart	0·094±0·01	16·6± 0·6	1·6
Diaphragm	0·045±0·01	25·7± 0·6	1·2
Spleen	0·236±0·04	10·2± 1·5	2·4
Lean‡	0·058±0·01	3226·0±81·7	186·5
Stomach	0·267±0·03	28·6± 1·8	7·6
Large intestine	0·385±0·01	46·9± 4·0	18·1
Small intestine	0·231±0·03	87·9± 1·6	20·3
Brain	0·475±0·27	7·0± 0·3	3·3
Lung	0·177±0·02	33·7± 2·2	6·0

• Mean of three animals ± standard error.
† Mean of six animals ± standard error.
‡ Mean of FSR for longissimus dorsi (0·040) and gastrocnemius (0·076) muscles.

Fractional synthetic rates (FSR) for liver and muscle are higher than those found by Garlick *et al.* (1976) using 70 kg pigs. The quantity of protein synthesized per day in individual tissues and organs was calculated from the FSR and the respective protein mass (related to a 24 kg pig).

Although excluding protein synthesized in hair, skin, blood and trotters, the sum of synthesis in individual tissues and organs gives 291 g of protein synthesized per day in the whole pig. This compares with a flux value of 300±23·7 g/d derived from the specific activity of [^{3}H]lysine at plateau in the blood of infused pigs. Dietary protein absorbed was 170·8±4·0 g/d and protein deposition amounted to 101±4·8 g/d. Relative to the 70 kg pig, these animals show a high efficency of protein synthesis as absorbed protein is 57% of flux and the ratio of synthesis to deposition approximates to 3:1.

Garlick, P. J., Burk, T. L. & Swick, R. W. (1976). *Am. J. Physiol.* **230**, 1108.
Waterlow, J. C. & Stephen, J. M. (1976). *Clin. Sci.* **33**, 489.

Diurnal variations in protein metabolism in man. By P. J. GARLICK, G. A. CLUGSTON, R. W. SWICK, I. H. MEINERTZHAGEN and J. C. WATERLOW, *Department of Human Nutrition, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT*

The rates of protein synthesis and oxidation at different times during the day were measured in seven obese women by continuous intravenous infusion of [1-^{14}C]leucine for 24 h. During the first 12 h of infusion the daily ration of food (85 g protein plus maintenance energy) was given hourly in 12 equal portions and during the remaining 12 h the patient fasted. Blood samples were taken at hourly intervals for measurement of free-leucine specific activity. In addition the rate of excretion of radioactivity in respiratory carbon dioxide was assessed regularly by measurement of the specific activity of expired CO_2 combined with measurement of CO_2 production rate by means of a ventilated hood. The rates of leucine flux, protein synthesis and leucine oxidation were calculated as described previously (James *et al.* 1976). The rates for leucine were converted to corresponding rates for protein on the assumption that protein contains 8% leucine.

During the feeding period plateau specific activity in both plasma leucine and respiratory CO_2 was reached after about 6 h and maintained until after the last hourly portion of food had been taken. The rates of flux, protein synthesis and protein oxidation calculated from this plateau period are shown in the Table (day-time). At the beginning of the fasting period there was an abrupt rise in the specific activity of plasma leucine to a new plateau followed by a fall in the specific activity of respiratory CO_2. The rates corresponding to the plateau values observed in the last 6 h infusion are also shown in the Table (night-time). Total flux, synthesis and oxidation were calculated on the assumption that the day-time and night-time values were maintained constant for the whole 12 h period.

Rates of flux, protein synthesis and oxidation (mean ± SD) measured at two different times during the day

	Day-time (g/h)	Night-time (g/h)	Total (g/d)
Flux	16·35±0·82	9·85±2·17	314±27
Synthesis	10·90±0·91	7·78±1·85	224±31
Oxidation	5·45±0·95	2·07±0·48	90±12

The rate of protein synthesis at night was 71% of the day-time value and protein oxidation at night was reduced to 38% of the rate during the day. Since Golden & Waterlow (1977) observed no change in protein metabolism over 24 h when food was given continuously, the diurnal cycle we have observed must result from the discontinuous intake of food.

Golden, M. H. N. & Waterlow, J. C. (1977). *Clin. Sci. Mol. Med.* 53, 277.
James, W. P. T., Garlick, P. J., Sender, P. M. & Waterlow, J. C. (1976). *Clin. Sci. Mol. Med.* 50, 525.

The digestion of dietary protein and synthesis of bacterial protein in the rumen in sheep given silages prepared with the addition of formic acid. By N. C. KELLY, P. C. THOMAS and D. G. CHAMBERLAIN, *The Hannah Research Institute, Ayr KA6 5HL*

Sheep fitted with ruminal cannulas and simple cannulas in the proximal duodenum and terminal ileum were used in two experiments to study the digestion of diets containing ryegrass silages prepared with the addition of formic acid (2·3 l/t of an 850 g/l solution). Three of the diets consisted solely of silage and these are considered here. The three silages were a wilted spring silage (S) and two direct-cut silages made early (E) or late (L) in the autumn from a regrowth sward. Six animals were used to study diet S and five for each of diets E and L. In each case the animals were given food twice daily at which times they were also given paper impregnated with chromic oxide which was used as an indigestible marker. The average rates of feeding for the three diets (g organic matter/d) were 586 (S), 612 (E) and 644 (L).

All three of the silages were highly digestible. Coefficients for organic matter (OM) digestibility were 0·70 (S), 0·82 (E) and 0·75 (L). Of the OM digested 65·2% (S), 72·0% (E) and 74·3% (L) 'disappeared' before the duodenum. The nitrogen contents of the silages (g N/kg dry matter) were 23·4 (S), 31·9 (E) and 22·1 (L) and the corresponding values for the proportion of the N in non-protein form were 64·4, 39·5 and 53·8%. N intakes (g/d) were 14·9 (S), 21·7 (E) and 15·6 (L) and the N passing to the duodenum (g/d) was 15·6±0·6 (S), 17·1±1·2 (E) and 17·0±0·8 (L). Of this N, ammonia accounted for 1·0–1·4 g/d and bacterial N, estimated using α-ε-diamino-pimelic acid as a marker, for 4·3–6·6 g/d. Assuming an endogenous secretion of N into the abomasum of about 1 g/d (Harrop, 1974) these results were adjusted to give duodenal flows of undigested dietary N plus protozoal N (g/d) of 5·9 (S), 9·5 (E) and 8·1 (L). The amount of protozoal N is probably small (Weller & Pilgrim, 1974) and the relationship between the adjusted flows and the dietary intakes of true protein N (5·3, 13·1 and 7·2 g/d) indicates that the dietary true protein is poorly degraded in the rumen. This may be a factor contributing towards the low rates observed for bacterial protein synthesis, 2·5 (S), 1·2 (E) and 1·6 (L) g bacterial N/100 g OM apparently digested in the stomach.

Harrop, C. J. F. (1974). *J. agric Sci., Camb.* **83**, 249.
Weller, R. A. & Pilgrim, A. F. (1974). *Br. J. Nutr.* **32**, 341.

General Practitioners and nutrition: a study in the Leeds and Bradford Health Authorities. By P. FIELDHOUSE and A. E. DE LOOY, *School of Health and Applied Sciences, Leeds Polytechnic, Leeds LS1 3HE*

Nutrition has a major role in preventive medicine. The General Practitioner, by the nature of his position in the community, is often responsible for interpreting nutritional knowledge to the public and for giving dietary advice. A survey was devised to investigate the nutritional knowledge held by the General Practitioner, his experience of nutritional problems amongst his patients and his perceived needs for further nutritional information.

Questionnaires were sent to 400 General Practitioners in the Leeds and Bradford Area Health Authorities. A final response of 72% was obtained. Preliminary analysis of the results shows that 55% of the General Practitioners considered that they had received some formal education in nutrition. To the question 'What topics could be most usefully included in a course on applied/clinical nutrition for GPs?' the response was: general nutrition and requirements for special groups of the population e.g. the elderly (35%), treatment of obesity (25%) and the role of diet in prevention and treatment of circulatory disorders (13%). The General Practitioners also indicated that several types of support and information services would be of use to them. First choice by 40% of the General Practitioners was the presence of a dietitian at the surgery or group practice. Access to a reliable nutrition information source (22%) was also highly rated.

Further analyses will be presented. Clearly there is a recognized need for more information on nutrition in health and disease, but the problem is how is this information to be effectively supplied.

This study was generously supported by the Medical Research Council.

Personality and food preferences. By HELEN L. MURRAY and R. H. J. WATSON, *Department of Food Science and Nutrition, Queen Elizabeth College, London W8 7AH*

The assessment of food preferences is thought to be an important predictor of patterns of food consumption in societies such as ours. In an effort to increase our understanding of the food likes and dislikes of different individuals, we have made a study of the relationships between personality variables and the rating of different foods on a five-point scale from 'like very much' to 'dislike very much'. We have investigated what Eysenck (1947) believes to be the two major personality dimensions: Extraversion and Neuroticism. Our aim was to determine whether extraverts and introverts differed in their food ratings and whether there was a difference between the food ratings of those with low and high Neuroticism scores.

The study was carried out on 53 female and 47 male postgraduate students at Queen Elizabeth College, University of London in mid-1977. The subjects filled in the Eysenck Personality Inventory (which measured Extraversion and Neuroticism) and rated each of the 21 foods on a printed questionnaire. The foods were selected on the basis of their nutrient content and flavour. Extraversion and Neuroticism scores were correlated with the food ratings.

The subjects with high Neuroticism scores did not differ significantly in their numbers of food likes and dislikes from those with low scores on this scale. These findings are not in accord with those of earlier investigators (Wallen, 1945; Gough, 1946) who showed that groups of neurotic males had significantly more aversions on a list of foods than did control groups. However, in our study differences were discovered in relation to nutrient content.

No previous studies of the food ratings of extraverts and introverts have been published. The male and female introverts in our study were found to have significantly more food dislikes ($P<0{\cdot}1$) than the extraverts. In addition, we found that extraverts and introverts differed in their ratings when nutrient content was considered. The possible relevance of these findings to the susceptibility of individuals to obesity is discussed.

Eysenck, H. J. (1947). *Dimensions of Personality*. London: Routledge and Kegan Paul.
Gough, H. G. (1946). *J. abnorm. soc. Psychol.* **41**, 86.
Wallen, R. (1945). *J. abnorm. soc. Psychol.* **40**, 77.

The diabetic diet: nuisance or necessity? By E. ANNE WILSON, DOROTHY CLARKE and D. R. HADDEN, *Metabolic Unit, Royal Victoria Hospital, Belfast*

As part of a wider prospective study of maturity onset diabetes, (Hadden *et al.* 1975) the response to intensive dietary management was monitored in a selected group of 58 patients attending the Diabetic Clinic over a period of 3 years. None of the 58 patients received oral hypoglycaemic agents or insulin.

The patients were seen routinely by the dietitian at each visit to the hospital and rated according to the estimated degree of dietary adherence. Exclusion of sucrose and sucrose-containing foods was the main dietary modification with a corresponding reduction in the energy value of the diet to given mean values of 6000 kJ, 148 g carbohydrate, 70 g protein and 64 g fat.

Mean observations (±SD) at selected time intervals are shown:

Time (months)	Weight (kg)	Fasting blood sugar (mmol/l)	Fasting plasma triglyceride (mmol/l)	Fasting plasma cholesterol (mmol/l)
0	81·0±14·0	10·4±3·4	2·5±2·1	6·2±1·3
6	72·3±12·1	7·0±2·0	1·8±1·1	6·4±1·6
12	71·4±10·3	7·2±2·0	1·5±0·6	6·2±1·2
24	71·1± 9·0	7·5±1·7	1·8±1·5	6·2±1·6
36	70·0±11·1	7·9±2·8	1·6±1·1	6·4±1·2

Good control of the metabolic abnormalities in the majority of patients with maturnity onset diabetes is possible by an acceptable degree of energy restriction without other medication. This study further analyses this control in terms of the dietitian's assessment of dietary adherence. The numerical grading of dietary adherence allowed classification into groups of 'good', 'fair' and 'poor' dieters. Those classified as 'good' dieters were less overweight (73·2 kg) at diagnosis although equally hyperglycaemic (10·3 mmol/l) they achieved a more normal weight with lower blood sugar and plasma triglyceride than did the 'poor' dieters. The plasma cholesterol did not change in any group.

Hadden, D. R., Montgomery, D. A. D., Skelly, R. J., Trimble, E. R., Weaver, J. A., Wilson, E. A. and Buchanan, K. D. (1975). *Br. Med. J.* 3, 276.

Immunological changes associated with the obob (obese) genotype. By C. J. MEADE, J. SHEENA and J. MERTIN, *Clinical Research Centre, Harrow*

Obese mutant mice (C57BL/6J obob) have been widely used as animal models for both obesity and diabetes mellitus. We find they have impaired cellular immunity, as demonstrated by reduced ability to react a contact sensitizing agent (picryl chloride), reduced ability of female mice to reject male skin grafts, and reduced generation of cytotoxic cells following in vivo sensitization with a DBA2 mastocytoma. In the common environment of a C57BL/6J × DBA/2JF$_1$ recipient, 'obese' and 'lean' spleen cells are equally able to mount a graft versus host reaction. Generation of cell-mediated cytotoxicity against DBA2 alloantigens in vitro is not impaired. The simplest interpretation of these results is that the obob genotype does not produce an irreversible functional change in those cells involved in the generation of cellular immune responses, but rather produces an environment in which lymphocytes are less able to react to alloantigens.

Hallberg *et al.* (1976) reported a similar discrepancy between alterations in in vitro and in vivo immune responses following treatment of obese patients by jejuno-ileal shunt. Our work emphasizes the importance of measuring in vivo parameters when studying immunity in diabetes and obesity.

Comparison of cell-mediated cytotoxicity following in vivo or in vitro sensitization

	Percentage corrected lysis at attacker:target ratio 5:1							
	12–14 d after in vivo sensitization with DBA2 mastocytoma				4 d after in vitro sensitization with irradiated DBA2 mastocytoma			
	Exp 1		Exp 2		Exp 1		Exp 2	
Source of cytotoxic responder cells	I	II	I	II	I	II	I	II
Obese mice (C57BL/6J obob)	7·2 (±2·5)	8·3 (±3·1)	23·2 (±2·0)	21·0 (±3·7)	31·1 (—)	30·2 (±2·4)	38·8 (±0·9)	27·0 (±0·5)
Lean littermates (+/ob or +/+)	21·4 (±4·8)	17·2 (±2·1)	38·7 (±6·8)	43·1 (±9·3)	20·9 (±1·0)	27·1 (±6·6)	36·9 (±3·6)	39·9 (±5·9)
Pure breeding age and sex matched lean mice (C57BL/6J+/+)	40·6 (±2·8)	31·6 (±3·8)	35·7 (±5·3)	53·1 (±7·4)	14·1 (±0·3)	23·8 (±3·1)	39·3 (±4·2)	45·2 (±1·5)

Kill was measured in quadriplicate at 3 attacker:target cell ratios, and percentage corrected lysis (as defined by Simpson *et al.* 1975) was determined from a 3 fit regression analysis (± one standard error).

Hallberg, D., Nilsson, B. S. & Backman, L. (1976). *Scand. J. Gastroent.* 11, 41.
Simpson, E., Gordon, R., Taylor, M., Mertin, J. & Chandler, P. (1975). *Eur. J. Immunol.* 5, 451.

Comparison of the effects of carrageenins and Danish agar on the colon of guinea-pigs. By J. WATT and A. J. MARCUS (Introduced by G. A. J. PITT), *Department of Pathology, University of Liverpool, Liverpool L69 3BX*

Carrageenin derived from the red seaweed *Eucheuma spinosum* is known to produce ulceration of the large bowel in a variety of laboratory animals when supplied in the drinking fluid (Marcus & Watt, 1969). In a comparative study, we have investigated the effects on the colon of guinea-pigs of carrageenins obtained from Chondrus and Gigartina species and Danish agar from Furcellaria.

Adult male albino guinea-pigs (mean body-weight 485 g) were fed a cube diet (R. G. P. Dixon & Sons Ltd, Ware) containing additional vitamin C. They were randomized into three groups of twelve animals each, housed four animals per cage. Two groups received as drinking fluid over a period of 4–8 weeks an aqueous solution (30 g/l) of carrageenin extracted from *Chondrus crispus* or *Gigarina acicularis*, the third group an aqueous solution (30 g/l) of Danish agar (Furcellaran). The seaweed extracts were first degraded by acid treatment to reduce the viscosity. Allowing for spillage from drinking bottles, the average daily consumption per animal of carrageenins and Danish agar was less than 5·8 (Chondrus), 5·5 (Gigartina) and 5·9 mg/g body-weight, respectively. A control group of animals received water only as drinking fluid.

Animals were killed by diethyl ether anaesthesia, six in each group at the end of 4 weeks, the remainder at the end of 8 weeks. The large intestine was emptied of faeces and carefully examined by transmitted light.

At the end of 8 weeks, the incidence of colonic ulceration in the three groups was twelve out of twelve, twelve out of twelve and eight out of twelve respectively in the Gigartina, Chondrus and Furcellaran fed animals. The severity of damage was least in the animals fed Furcellaran and greatest in the animals fed carrageenin of Gigartina origin. An increase in the severity of ulceration at the end of 8 weeks compared with 4 weeks was observed only in relation to the animals fed Gigartina-derived carrageenin. No colonic ulcers were found in any of the control animals.

These results indicate that carrageenins derived from Chondrus and Gigartina species, as well as Danish agar, products commonly used as food additives, cause ulceration of the colon in guinea-pigs.

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Diet and faecal flora: a comparison of rural Northern Nigeria and London, UK. By P. BORRIELLO, B. DRASAR and A. TOMKINS, *Bacterial Metabolism Research Laboratory, Colindale, London and Departments of Microbiology and Human Nutrition, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT*

Intestinal cancer is more common in developed than developing countries and there are marked differences in faecal bacteria of adult subjects from such communities particularly with regard to the incidence of bacteria capable of dehydrogenating steroids into possible carcinogenic compounds (NDH + ve clostridia). However, it is uncertain whether such differences are the result of diet or environment, although strong correlations have been shown internationally between dietary intake and cancer incidence (Drasar & Jenkins, 1976).

Attempts at alteration of faecal flora by dietary change are unsuccessful, in the adult at least. Changes in faecal flora do occur in the infant, however, during change from breast to bottle feeding (Bullen *et al.* 1977). The effect of the other variable, environmental contamination, on the establishment of the human faecal flora is unknown.

Faecal samples from healthy subjects in London (adults, weaned children age 2–4 years and infants fed solely on breast milk) were compared with samples from equivalent age groups in rural Northern Nigeria.

Breast-fed infants in both communities had similar carriage rates of Bacteroides spp. and NDH + ve clostridia. After weaning numbers with Bacteroides spp. increased in both groups (see table). Only 5% of Nigerian children had NDH + ve clostridia but 25% of the UK children carried these bacteria. The difference in adults was even greater; no adult Nigerian carried NDH + ve clostridia whereas 33% of UK adults passed these bacteria. There were six times as many UK, as Nigerian, subjects who carried Bacteroides spp.

Comparison of faeces obtained from breast-fed infants, in London, UK and Nigeria demonstrated that breast feeding overrode any effect of environment, numbers of bifidobacteria and *Escherichia coli* were the same. A similar bacterial flora producing the same volatile fatty acid metabolic products was demonstrable in each group.

	% Carriage			Clostridia $\log_{10}$ g^{-1} wet wt		
	NDH + ve	Bacteroides	Clostridia	Total	Lecithinase + ve	Lecithinase − ve
Nigerian infants	4	11	85	2·3±1·3	1·5±1·1	1·4±1·6
UK infants	0	28	86	3·5±2·4	2·7±1·5	3·2±2·5
Nigerian children	5	43	100	5·2±1·6	5·1±1·7	0·7±1·8
UK children	25	66	100	4·6±1·4	4·1±0·8	3·1±2·8
Nigerian adults	0	16	100	6·0±1·5	5·9±1·5	1·1±2·0
UK adults	33	100	100	5·2±1·1	—	5·1±1·0

Only 6·6% British adults carried lecithinase + ve clostridia in numbers greater than 10^4 g^{-1} wet wt, compared with a value of 80% for Nigerian adults.

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Biochemical responses of women with low storage iron to a sustained release preparation of oral Fe. By E. FAIRHURST, T. L. C. DALE and B. D. RIDGE, *Beecham Products Research Department, Randalls Road, Leatherhead, Surrey KT22 7RX*

The effectiveness of a sustained release oral ferrous sulphate preparation in alleviating anaemia has been demonstrated by Myers & Robinson (1971). We have studied the ability of the same preparation to replenish storage iron.

Having determined the Fe status of 125 working women (Fairhurst *et al.* 1977), we selected for trial those 42 subjects with the lowest plasma ferritin concentrations. Seventeen were Fe deficient (plasma ferritin below 10 μg/l) of whom five were anaemic (haemoglobin below 12 g/100 ml blood). A total of eight cases of anaemia were detected, of whom five were also Fe deficient.

Each subject ingested the equivalent of 130 mg elemental Fe daily for 16 weeks. For the first 9 weeks, medication was supervised. Haemoglobin and plasma ferritin concentrations (plus transferrin saturation as a check on the latter) were determined before supplementation and periodically during treatment. Mean initial values and the responses to treatment are shown in Table 1.

Table 1. *Effect on iron status of regular daily ingestion of a sustained release oral Fe preparation*

(Results expressed as mean values for subjects in the group; numbers of individuals in parentheses)

Biochemical parameter	Group	Period of treatment (weeks) 0	1	2	4	9	16
Plasma ferritin (μg/l)	All subjects (42)	12·0	35·2	33·3	37·9	46·5	49·3
	Iron deficient (17)	5·9	43·7	35·4	44·3	40·5	43·3
	Anaemic (8)	8·4	55·3	48·6	42·3	53·3	46·9
Haemoglobin (g/100 ml)	All subjects (42)	13·4	13·0	13·1	13·3	13·8	13·6
	Iron deficient (17)	12·4	12·1	12·6	12·9	13·7	13·6
	Anaemic (8)	11·1	11·8	12·4	12·6	13·7	13·5

Within one week of starting treatment, 15 of the 17 Fe-deficient subjects were no longer deficient as judged by plasma ferritin response. That this very rapid response represented a real rise in Fe stores is supported by the corresponding rises in saturation of transferrin and by the fact that plasma ferritin remained elevated 16 weeks after cessation of treatment. All Fe deficiency was eliminated within 4 weeks and after 9 weeks of treatment no subject was anaemic.

There was no evidence of Fe overload as judged by any of the measures.

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Rickets and vitamin D metabolism in primates. By A. W. M. HAY, M. DARLING and G. WATSON, *Nuffield Laboratories of Comparative Medicine, Zoological Society of London, Regent's Park, London NW1 4RY*

For many years skeletal disease in primates presented considerable animal management problems for staff in zoos. However, the introduction in recent years of purified 'balanced' diets in pelleted form has resulted in a marked reduction in the incidence of rickets or osteomalacia in these animals.

It has been shown that in New World (NW) monkeys vitamin D_2 is less efficient than vitamin D_3 where calcium absorption (Hunt *et al.* 1967*a*) or the prevention of metabolic bone disease (Hunt *et al.* 1967*b*) is the criterion. Metabolic bone disease is not common, however, in Old World (OW) primates maintained on diets where vitamin D is only provided as vitamin D_2 (Hunt *et al.* 1967*b*).

We have also found vitamin D_2 to be less efficient than vitamin D_3 in NW primates. Bone lesions in a rachitic Capuchin monkey (*Cebus*) healed when the animal received vitamin D_3 (250 IU/1·5 kg body-weight per week) intramuscularly for 10 weeks; an equivalent dose of vitamin D_2 in a second rachitic Capuchin brought about no healing. Rickets in Old World Patas monkeys (*Erythrocebus patas*) was successfully treated with either vitamin D_3 or D_2 (150 IU/kg body-weight per d) administered as a daily dose in the diet for 7 weeks, confirming the observations of Hunt *et al.* (1967*b*) that both vitamins appear to be equally efficacious in preventing bone disease in OW primates. These vitamin D regimens are too high for normal OW primates. They may even be too high for treating rachitic animals. In subsequent trials we have successfully treated rachitic Patas monkeys with lower oral doses of vitamin D_3 (23 IU/kg body-weight per d).

Many commercial formulations of primate diet have vitamin D_3 concentrations which range from 2200–11400 IU/kg dry weight. We have found that a primate diet (Herbert, 1971) with the vitamin D_3 content lowered to 550 IU/kg dry weight is still adequate for healthy non-adult primates. Where pelleted diets are used to provide up to 75% of the energy intake of primates it may be useful, therefore, to reassess some of those with higher vitamin D formulations.

We have investigated vitamin D_3 metabolism in Patas and Capuchin monkeys using isotopically labelled vitamin. We found the metabolism of vitamin D_3 in the Patas monkey to be similar to that reported in man (Mawer, 1977). In the Capuchin monkey the metabolism of vitamin D_3 varied according to the state of vitamin D nutrition; 1,25-dihydroxycholecalciferol was not observed in vitamin D-replete animals but only in those which were vitamin D deficient. Capuchin monkeys appear to have more 25,26-dihydroxycholecalciferol in blood than Patas monkeys.

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Effect of duodenal cannulation on digesta flow in the preruminant calf.

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Although suitably sited cannulas have often been used to study intestinal function in the pre-ruminant calf there is very little information on the effect of such cannulation on digesta movement and secretion in the tract. To obtain such information calves were maintained on cow's milk as their sole diet except when they were given experimental feeds. At 3 weeks of age they were fitted with simple abomasal cannulas and, at the same time or 5 weeks later, with re-entrant duodenal cannulas also. Experiments began not sooner than 15 d after an operation and were then done at intervals of 2–3 d. An experimental feed (2·5 l) containing only glucose and minerals ('Mineral feed', Smith & Sissons, 1975) with polyethylene glycol (PEG) as a marker was given at 09.30 hours by direct infusion into the abomasum. Estimates of the volume of abomasal contents were made at intervals of 0·5–1·0 h by dye (phenol red) dilution (George, 1968). Changes in PEG concentration were also observed. If, in an interval when the volume of abomasal contents changed from V_1 to V_2 and PEG concentration from C_1 to C_2, it is assumed that the average PEG concentration in digesta flowing from the abomasum was $(C_1+C_2)/2$ then the volume of digesta (E) passing into the duodenum is $2(V_1C_1-V_2C_2)/C_1+C_2$ and the volume secreted into the abomasum (S) is V_2+E-V_1. Values were compared for different conditions in the duodenum. When an open duodenal cannula was used, digesta was measured and returned automatically as described by Smith & Sissons (1975). Mean values (l) with standard errors (six and four determinations for age ranges 30–60 and 80–120 d respectively) for E and S estimated between 0–1 and 2–3 h after feeding were

Calf no.	Age range (d)	Duodenal cannula	E (0–1 h) Mean	E (0–1 h) SE	E (2–3 h) Mean	E (2–3 h) SE	S (0–1 h) Mean	S (0–1 h) SE	S (2–3 h) Mean	S (2–3 h) SE
1	30–60	closed	1·17	0·10	0·58	0·08	0·20	0·05	0·17	0·03
2	30–60	absent	1·30	0·12	0·63	0·09	0·35	0·06	0·17	0·03
	80–120	closed	1·39	0·15	0·63	0·10	0·36	0·06	0·18	0·04
		open	1·15	0·10	0·57	0·04	0·31	0·04	0·12	0·02
		open	0·98•	0·06	0·59•	0·09				

• Determined by direct collection from duodenal cannula.

Estimates of duodenal flow based on marker dilution in the abomasum gave results very similar to those obtained by direct measurement. They were typical of values observed in other calves (Smith & Sissons, 1975).

Duodenal flow and secretion into the abomasum did not appear to be significantly affected by inserting a re-entrant cannula into the duodenum or by opening that cannula provided digesta was returned promptly.

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The effects of a low-protein diet on milk yield and plasma metabolites in Friesian heifers during early lactation. By J. D. OLDHAM, W. H. BROSTER and J. W. SIVITER, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The aim of this experiment was to examine the effects of deliberate underfeeding of protein to dairy cows during the first 10 weeks of lactation.

Sixteen first-calf Friesian heifers were used. Feed (kg/d) was increased stepwise until day 8 after calving and was then constant at 3·6 kg hay + 10·8 kg concentrates/d for 62 d. Up to day 10 after calving all animals received the same concentrates (MP, 400 g barley, 200 g soya-bean meal, 400 g (flaked maize + sugar beet pulp + supplements) per kg concentrates). On day 11 they were split into 2 groups of 8 and changed abruptly to either high protein concentrates (HP) (MP with 200 g barley replaced by 200 g soya-bean meal) or low-protein concentrates (LP) (MP with 200 g soya-bean meal replaced by 200 g barley).

Throughout the trial jugular venous blood samples were taken at 5 d intervals between 09.30 and 10.30 hours. Digestibility was measured in six animals from each group over 10 d between the 5th and 9th week of lactation. Bulk milk samples were analysed twice per week for fat (F), protein (P) and lactose (L) content. The heifers were weighed three times weekly.

	Intake (kg/d)		Digestibility			Yield (kg/d)				Live wt loss	Plasma		
Diet	DM	CP	DM	NDF	OM	Milk	P	L	F	(kg)	Urea (mM)	Alb (g/l)	Mg (mM)
LP	11·81	1·26	0·67	0·44	0·68	20·9	0·61	1·00	0·76	48·6	1·09	36·8	0·89
HP	12·14	2·71	0·77	0·62	0·78	24·4	0·73	1·16	1·07	23·8	8·49	39·4	0·96
SE of difference	0·095	0·014	0·006	0·014	0·006	1·04	0·026	0·048	0·066	11·13	0·442	0·93	0·031
Difference	••	•••	•••	•••	•••	••	•••	••	•••	••	•••	•	+

+ $P<0·1$ • $P<0·05$ •• $P<0·01$ ••• $P<0·001$.

NDF, Neutral detergent fibre (Van Soest & Wine, 1967), DM dry matter, OM organic matter.

LP reduced DM digestibility due almost entirely to reduced fibre digestion. Because of this there were some hay refusals and DM intakes were slightly lower for LP. Milk, milk protein and milk lactose yields for HP were 116–119% of those for LP but milk fat yield was proportionately much greater for HP (141% of LP). LP animals lost more weight than HP.

Total protein (TP), packed-cell volume, haemoglobin, Ca, Na and K in plasma were similar for both groups but urea, albumin (Alb) and Mg were lower for LP. TP and albumin rose gradually up to week 5 in both groups.

Changes in energy supply from the gut, due mainly to altered fibre digestion, were responsible for many of these differences but these results together with those of Stead & Oldham (1978) suggest that low protein feeding may interfere with milk fat synthesis at some point. The influence of these treatments on subsequent lactation will be discussed.

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The effects of low- and high-protein diets upon the concentrations of plasma low density lipoproteins, in Friesian heifers throughout lactation. By D. STEAD and J. D. OLDHAM, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

In the dairy cow, the plasma lipoproteins of density <1·06 g/ml transport triglycerides to the mammary gland for milk fat synthesis (Glascock & Welch, 1974). The varying demands for, and supply of, milk fat precursors at different states of nutrition and lactation may affect the concentrations of these lipoproteins.

In the present experiment, the plasma concentrations of low density lipoprotein-1 (LDL_1, density 1·019–1·039 g/ml) and of low density lipoprotein-2 (LDL_2, density 1·039–1·060 g/ml) were determined in late pregnancy and throughout lactation in nine Friesian heifers, four given high-protein concentrates (HP) and five given low-protein concentrates (LP). The diets are described in the previous paper (Oldham *et al.* 1978), except that at the 10th week of lactation, all cows were gradually (over a period of 3 weeks) changed to, and thereafter maintained on, a moderate protein diet.

Plasma was adjusted to density 1·06 g/ml with KBr, ultracentrifuged and the supernatant electrophoresed on polyacrylamide gels with albumin as internal standard. Protein bands were assayed by densitometry. Concentrations are in arbitrary units relative to albumin.

	Period after calving (d)	20	30	40	50	60	65
LDL_1	Low protein	53	143	272	424	473	543
	High protein	15	19	53	75	95	86
	SE difference of means	13	34	60	89	49	54
	Significance of difference	•	••	••	••	•••	•••
LDL_2	Low protein	102	105	105	90	84	84
	High protein	75	99	93	91	85	88
	SE difference of means	12	21	51	24	16	22
	Significance of difference	NS	NS	NS	NS	NS	NS

NS, not significant $P>0·05$ • $P<0·05$ •• $P<0·01$ ••• $P<0·001$.

In all cows LDL_2 was low in pregnancy and early lactation, and LDL_1 was either very low or undetected. During the first 65 d of lactation, LDL_1 increased markedly in the cows on the LP diet, but much less so in those on the HP diet. LDL_2 increased during the first 20 d of lactation but then remained constant for the next 50 d and also for the period 125–200 d. LDL_2 was not affected by the diet.

After the change to moderate protein diet, LDL_1 initially decreased in cows previously on LP diet, and initially increased in those previously on HP diet. From 125–300 d LDL_1 was not related to previous dietary treatment.

The high concentrations of LDL_1 in cows on the LP diet were accompanied by reduced milk fat yield and suggest an inefficient uptake of this precursor for milk fat synthesis.

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