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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Seventh Meeting of the Nutrition Society was held at The Clinical Research Centre, Harrow, Middlesex HA1 3UJ on Monday, 12 *September,* 1977, *when the following posters were presented:*

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The prevalence of obesity in working populations in London. By MARGARET ASHWELL and W. R. S. NORTH, *Division of Clinical Investigation, Clinical Research Centre, and MRC–DHSS Epidemiology and Medical Care Unit, Northwick Park Hospital, Harrow, Middlesex*

Height, weight and skinfold thicknesses at four sites (forearm, triceps, subscapular and suprailiac) have been measured in 1329 white men and 577 white women employees of four working populations in North-West London; age and social class were obtained at interview.

In this population, 30·8% of the men and 38·6% of the women were more than 20% above their ideal weight for height (according to the Metropolitan Life Insurance Tables). Only 36·9% of the men and 28·8% of the women had weights within ±10% of their ideal weight for height.

The results in the table show that obesity, whether defined as W/H^2 or the sum of skinfolds, increased with age in both sexes. The effect was seen in all social classes.

	Age	*n*	W/H^2	Σ skinfolds (mm)
Men	18–29	245	23·64±2·98	38·1±17·7
	30–49	587	25·06±3·10	42·2±17·5
	50–64	497	25·59±3·08	43·3±16·8
Women	18–29	100	22·27±2·45	44·2±12·9
	30–49	245	24·24±3·78	56·7±20·8
	50–59	232	25·38±3·60	62·7±23·7

Mean values ± SD.

The relationship between obesity and social class is complex, and is affected by age and the definition of obesity used. Considering all age groups together, there was no apparent effect of social class on obesity in men, though the prevalence of obesity tended to be lower in younger men of Classes I and II than of Classes IV and V. For women, W/H^2 was higher in the lower than the upper social classes in all age groups, but total skinfolds did not show such a clear relationship. Further analysis of our results shows that in considering the relationship between social class and obesity, it is also necessary to take smoking habits into account.

Our results demonstrate a higher prevalence of obesity than in a previous survey in London (Baird *et al.* 1974); they confirm the results of other studies on the prevalence of obesity in different age groups.

Baird, I. M., Silverstone, J. T., Grimshaw, J. J. & Ashwell, M. A. (1974). *Ptactitioner* **212**, 706.

Morphological and metabolic site differences in human subcutaneous adipose tissue. By MARGARET ASHWELL, MERRIL DURRANT, SUSAN STALLEY and J. S. GARROW, *Division of Clinical Investigation, Clinical Research Centre, Watford Road, Harrow, Middlesex*

Overweight women often find that some fat sites are more resistant to dieting than others.

Nineteen women (W/H^2 ranging from 24·5 to 50·2) were studied in a metabolic unit for 3 weeks, protocol of which had been approved by the hospital ethical committee. Their mean daily energy intake was 778±65 kcal (3·26±0·27 MJ). On day 1 and day 21, they were photographed unclothed (Stalley & Garrow, 1975). At 9.00 hours on day 6, percutaneous needle biopsies of adipose tissue were taken from fasting patients at three sites: lateral arm, mid-abdomen and upper thigh. Fat cell mass (CM) was measured in fixed sections of adipose tissue (Ashwell *et al.* 1976). Adipose tissue from each site was incubated in Krebs-Ringer bicarbonate buffer at 37°. Dibutyryl cAMP (0·001 M) was present during the first hour of incubation for 10 subjects, and fresh buffer containing isoprenaline hydrochloride (IP) (0·001 mM) was present during the second hour for all subjects. Lipolysis was measured by estimating glycerol release into the medium.

There was no significant difference in CM for the 3 biopsied sites (arm CM=0·36±0·229 μg, waist CM=0·563±0·189 μg, thigh CM=0·571±0·235 μg). However, there was a large range of intra-patient variation in CM between sites. The mean coefficient of variation was 20, the minimum being 4 and the maximum 50.

IP and cAMP stimulated lipolysis were positively correlated with each other (r 0·856, $P<0·001$, n 29). cAMP stimulated lipolysis showed greater correlation with CM (r 0·559, n 29, $P<0·01$) than IP stimulated lipolysis (r 0·335, n 55, $P<0·05$). Stimulated lipolysis by cAMP and IP showed greater correlations with CM in the lateral arm site compared with other sites.

Site changes at the upper arm (SCA) and the maximum thigh (SCT) were calculated from the two side view photographs and expressed as the change in diameter measurement divided by the original measurement (×100%). SCA and SCT were ranked as I or II for each patient; rank I being assigned to that site showing the greater change. This ranking revealed a significant difference (rank I>rank II: $P<0·05$, n 16) in IP-stimulated lipolysis but not in respect to cAMP-stimulated lipolysis or CM.

We have, therefore, shown differences not only in adipose tissue cellularity at different fat sites in some individuals, but also in sensitivity to hormone-stimulated lipolysis.

This investigation had the approval of the Northwick Park Hospital Ethical Committee.

Ashwell, M. A., Priest, P., Bondoux, M., Sowter, C. & McPherson, C. K. (1976). *J. Lipid Res.* **17**, 190.

Stalley, S. & Garrow, J. S. (1975). *Rec. Adv. Obesity Res.* **1**, 66.

How a 'fat cell pool' hypothesis could account for the relationship between adipose tissue cellularity and the age of onset of obesity.

By MARGARET ASHWELL, MERRIL DURRANT and J. S. GARROW, *Division of Clinical Investigation, Clinical Research Centre, Harrow, Middlesex*

It has been suggested (Brook *et al.* 1972; Salans *et al.* 1973) that child-onset obese subjects are likely to have more fat cells than adult-onset obese subjects. We have measured fat cell mass in fixed sections of adipose tissue from three sites (lateral arm, mid-abdomen and upper thigh) in 46 women whose obesity index (W/H^2) ranged from 24·3 to 51·8. Age of onset of obesity (OA) was determined by interview and confirmed by photographs. Apparent total fat cell number (TCN) was obtained by dividing total body fat (estimated from body potassium) by average fat cell mass (CM). Standard linear regression analysis showed no significant correlation between OA and either CM or TCN. When the subjects were split into two groups; child onset (CO)=OA<18 years; adult onset (AO)=OA>18 years, there was no significant difference in TCN ($CO=9\cdot18\pm2\cdot49\times10^{10}$; $AO=8\cdot39\pm2\cdot78\times10^{10}$) or CM ($CO=0\cdot51\pm0\cdot16$ μg, $AO=0\cdot54\pm0\cdot14$ μg).

Splitting the subjects into two groups did, however, show an effect of OA on the relationship between the severity of obesity and adipose tissue cellularity. For the CO group, there was a strong positive correlation of W/H^2 with CM (r 0·662, $P<0\cdot001$, $n=23$) but a weaker correlation between W/H^2 and TCN (r 0·464, $P<0\cdot05$). For the AO group there was a strong positive correlation between W/H^2 and TCN (r 0·765, $P<0\cdot001$, $n=23$), but no significant correlation between W/H^2 and CM. The regression coefficients in the equations relating TCN to W/H^2 were significantly different for the CO and AO groups ($P<0\cdot05$).

One interpretation of our results is to assume that: (a) adipose tissue contains a 'fat cell pool' of mature and immature cells, and (b) that immature cells can only be recruited at times of energy surplus. In the CO group, where the degree of obesity is determined more by the size of the fat cells, immature cells were probably recruited early on and the severity of the obesity thereafter depended solely upon the extent of filling of cells. In the AO group, however, where the degree of obesity is determined more by cell number, immature fat cells are still available for recruitment as the degree of obesity increases.

A 'fat cell pool' hypothesis could therefore explain why some of the AO group as well as some of the CO group have an increased fat cell number.

This investigation had the approval of the Northwick Park Hospital Ethical Committee.

Brook, C. G. D., Lloyd, J. K. & Wolf, O. H. (1972). *Br. med. J.* ii, 25.
Salans, L. B., Cushman, S. W. & Weismann, R. E. (1973). *J. clin. Invest.* 52, 929.

Weight loss, resting metabolic rate, physical activity and body composition in obese women on a reducing diet. By J. S. GARROW, D. HALLIDAY, R. HESP, S. F. STALLEY and P. M. WARWICK, *Clinical Research Centre, Watford Road, Harrow, Middlesex*

Thirty-seven women weighing 93·1±22·2 kg were studied in a metabolic ward for 3 weeks on a protocol which had been approved by the hospital ethical committee. Their diet was designed to provide 3·34 MJ/d, but when allowance was made for food not eaten intake was 2·50 to 3·66 MJ (average 3·28 MJ). The patients' diet immediately before coming into hospital was assessed by questionnaire. All patients had measurements of resting metabolic rate by indirect calorimetry, total body potassium by gamma spectrometry, total body water by dilution of deuterium oxide, and nitrogen balance throughout the 3 weeks. Five patients had a programme of exercise on a treadmill which caused them to expend about 1·2 MJ extra daily.

Average rate of weight loss was 215±80 g/d. Rate of weight loss was best correlated with resting metabolic rate (r 0·80), which in turn was correlated with weight (r 0·88). When the relative contribution of lean tissue (calculated from K or water) and fat to metabolic rate was examined by stepwise regression, the estimated fat content of the patient made no significant reduction to the variance already explained by lean mass. The patients who took extra exercise lost less weight, and less K, than those not exercising, when allowance was made for the energy deficit in the two groups. There were 13 patients who gave a history of being on a reducing diet before admission, and compared with 13 who had not been on a reducing diet before admission, they lost significantly less weight (especially in the first week), less K, and showed a small fall in resting metabolic rate.

We conclude that weight loss on a fixed reducing diet is determined chiefly by the patient's resting metabolic rate, that there is an early phase associated with loss of lean tissue, and that exercise under the conditions of this study decreased weight loss and K loss.

Investigations into patient responses to feeding low- and high-energy foods. By MERRIL DURRANT and SHIRLEY MANN, *Clinical Research Centre, Harrow HA1 3UJ, Middlesex*

Durrant *et al.* (1977) have reported that during 8 h feeding trials patients did not associate a decrease in energy density of food intake with an increase in energy content of the food. A similar sequential trial was designed in order to ascertain whether real energy differences were discernible to patients under the same test conditions.

Ten overweight patients were tested on two pairs of two consecutive test days during a 21 d stay in a metabolic unit where they were undergoing a course of weight reduction. The protocol had been approved by the hospital ethical committee.

Paired days were arranged such that there was a 50% difference in energy intake and the volume of the diet was in reverse order to the energy content, thus the low-energy diet was given in high volume and vice versa. Low-energy days (9, 10) contributed 992 kJ in 474 g (A, density 2·09 kJ/g) and 1548 kJ in 367 g (B, density 4·20 kJ/g). The high-energy days (14, 15) contributed 1933 kJ in 769 g (C, density 2·51 kJ/g) or 2971 kJ in 594 g (D, density 5·00 kJ/g). The food consisted of soups, milkshakes and sandwiches, which were disguised in taste and texture so the energy differences were not obvious. Alternate patients were fed in the order of AB/DC or BA/CD. During the 8 h of the study, patients made five recordings of hunger (internal feelings) and desire to eat (mental signals) on separate 0–4 rating scales. At the end of each pair of days, patients were required to choose which day they associated with highest energy intake. Results were plotted on a closed sequential trial design (Armitage, 1960).

Out of nineteen patient choices, fifteen were for high energy–low volume and four were for low energy–high volume intake. The trial reached the upper significant boundary ($P<0.05$) showing that patients significantly associated high energy rather than high volume of intake with high energy intake. They also reported significantly lower hunger and drive scores associated with the high-energy level choices. It is concluded that under these circumstances, patients were able to detect energy differences in the food they were given.

Armitage, P. (1960). *Sequential Medical Trials.* Oxford: Blackwell Scientific Publications.
Durrant, M., Toft, R., Mann, S. & Garrow, J. S. (1977). *Proc. Nutr. Soc.* (In the Press.)

Can genetically transmitted obesity be ascribed to an adipose tissue defect? By C. J. MEADE[1], MARGARET ASHWELL[2], P. B. MEDAWAR[1], and C. SOWTER[3], *Departments of [1]Transplantation Biology, [2]Clinical Investigation and [3]Histopathology, Clinical Research Centre, Watford Road, Harrow HA1 3UJ, Middlesex*

Previously (Ashwell *et al.* 1977) we described a technique of transplanting 'lean' fat under one kidney capsule and 'obese' fat under the other kidney capsule of the same mouse. Grafts were removed after 1 month, and cell size measured in fixed sections. Using this technique, we showed host environment was the all important factor in determining final fat cell size in grafts from either obese mice (C57BL/6 J obob) or lean littermates. Here we report results from four more rodents (details in table). Lean littermates were the controls for the obese mouse mutants; for the hamsters the B10.4.22 strain was the lean control.

Obese rodent	No. of mice used for cell sizing		Body mass of recipient when killed (g)		Fat cell masses (μg)					
					Fat from 'lean' donor			Fat from 'obese' donor		
	lean	obese	lean	obese	Before transplant	After 1 month in lean recipient	After 1 month in obese recipient	Before transplant	After 1 month in lean recipient	After 1 month in obese recipient
Diabetic mouse C57BL/6J db/db	8	9	26·3 (±1·6)	38·4 (±2·1)	0·128	0·101 (±0·045)	0·765 (±0·180)	0·688	0·083 (±0·020)	0·677 (±0·186)
Yellow obese mouse C57BL/6J A_y/a	13	11	27·3 (±3·2)	40·7 (±8·7)	0·093	0·118 (±0·034)	0·449 (±0·171)	0·575	0·112 (±0·076)	0·368 (±0·138)
Adipose mouse C57BL/6J ad/ad	4	6	25·4 (±2·9)	45·2 (±3·9)	0·092	0·073 (±0·022)	0·602 (±0·134)	0·682	0·069 (±0·024)	0·519 (±0·104)
B10.4.24 hamster	7	7	91·0 (±8·9)	144·0 (±14·2)	0·101	0·117 (±0·025)	0·173 (±0·043)	0·406	0·098 (±0·024)	0·145 (±0·051)

(Mean values and standard deviations)

'Lean' fat transplanted into obese mice, or 'obese' fat transplanted into lean mice, underwent a significant change in fat cell mass. Comparison of grafts of the same fat tissue into 'obese' or lean recipient mice showed significant differences ($P<0.001$ in all cases). Graft cell size altered to that characteristic of the recipient.

'Lean' hamster fat cells transplanted into obese recipients increased in mass, but rarely reached a mass comparable to the recipient fat, perhaps because hamsters (unlike mice) frequently lost weight after operation. After 1 month the same fat cells grafted into lean or obese hamsters differed significantly in size ($P<0.05$).

Thus the environment of transplanted fat tissue, not its genetic origin, determined fat cell mass. There was no evidence for a defect in the fat tissue.

Costs of maintenance and growth in genetically obese (obob) mice.

By C. J. H. WOODWARD, P. TRAYHURN and W. P. T. JAMES, *MRC Dunn Nutrition Unit, Milton Road, Cambridge*

We have studied the metabolic efficiency of obob mice aged 4–10 weeks, since it is well recognized that hyperphagia is only one cause of their obesity. In an initial experiment obob mice were pair-fed to lean littermates eating ad lib., and the gross efficiency (energy deposition ÷ intake) was found to be 31·7±1·2% (±SEM) and 13·8±1·1% respectively ($P<0.001$); similar differences in gross efficiency have been noted by other authors (Welton *et al.* 1973). The cause of the difference, which could arise either from maintenance expenditure or the cost of growth, was investigated. Groups of lean and obob littermates were pair-fed at different intake levels and measurements made of energy intake, and of fat and protein deposition. Maintenance needs were calculated from the amount of diet required to keep animals of different weights in weight balance.

Energy utilization was expressed as $I=M+\alpha W$ (I is the daily energy intake, M the maintenance cost, and W the weight of protein and fat deposited), assuming that the costs of depositing fat and protein are similar (Pullar & Webster, 1977). The cost of growth (α) for lean and obob animals was 78·2±16·9 kJ/g and 77·8±8·7 kJ/g respectively, which suggests that the costs of fat and protein deposition were each unaltered in the mutant. Subsequent regression analysis showed that the cost of fat deposition in the obese mouse was 59·9±5·2 kJ/g, a value similar to the 61·5 kJ/g obtained for lean animals and to values reported by others (e.g. Pullar & Webster, 1977). The maintenance costs for the obob mice were, however, significantly lower than for the lean. The linear regressions were $M=1.07W+8.13$ (r 0·985; n 14) and $M=1.44W+13.85$ (r 0·944; n 12) for obob and lean animals respectively. The difference in maintenance costs at a weight of 30 g, for example, is 16·8 kJ/d which is sufficient for the deposition of an additional 0·28 g fat.

We conclude that the higher gross efficiency of obob mice pair-fed to lean littermates is due entirely to a reduction in the maintenance costs and not to changes in the efficiency of growth.

C.J.H.W. holds an MRC Research Studentship.

Pullar, J. D. & Webster, A. J. F. (1977). *Br. J. Nutr.* **37**, 355.
Welton, R. F., Martin, R. J. & Baumgardt, B. R. (1973). *J. Nutr.* **103**, 1212.

Use of 13-methyl-myristic acid as an indicator of adipose tissue turnover rates at various sites in rat and man. By P. G. PITTET and D. HALLIDAY, *Clinical Research Centre, Harrow* and R. A. KLEIN, *Molteno Institute, Cambridge*

The measurement of adipose tissue turnover in vivo using radioactivity-labelled precursors is ethically limited to animal studies; use of small amounts of a structurally-labelled fatty acid, occurring naturally in traces and not synthesized in the body, provides a method more applicable to clinical studies in man (cf. Campbell & Hashim, 1972). 13-methyl-myristic acid (13MeM), a branched-chain odd carbon fatty acid, was chemically synthesized and added to maize oil (approx. 1:9 w/w). Rats were weaned onto a controlled diet containing 10% w/w maize oil; after 3 weeks a diet with maize oil containing 13MeM was substituted and continued for another 3 weeks whereupon the diet was changed back to the original mixture. Groups of rats were sacrificed at weekly intervals throughout this dietary regime to follow the incorporation of 13MeM into, and subsequent loss from adipose tissue at various sites. The ratio, 13MeM:palmitate was determined by gas–liquid chromatography. Typical ratios for subcutaneous adipose tissue were 0·005, 0·066, 0·106 and 0·106 before and after 1, 2 and 3 weeks on the 13MeM diet, respectively, giving a half-life for incorporation rate of about 5 d. The direct incorporation of 13MeM was confirmed by GC-MS. The disappearance of 13MeM was also studied and typical ratios for subcutaneous adipose tissue were 0·085, 0·047 and 0·030, 1, 3 and 7 weeks after return to the original diet, showing a half-life for disappearance rate of about 22 d. Values obtained from other sites did not differ significantly with the exception of pericardiac fat, which incorporation rate was much slower and did not exhibit any plateau after 2 weeks on the 13MeM diet. A normal weight adult subject was fed 2 g 13MeM for 7 d and analysis of subcutaneous fat showed an unequivocal increase in the ratio 13MeM:palmitate. Samples of subcutaneous fat from three sites (arm, waist and thigh) of an obese subject given 2·5 g 13MeM for 14 d was investigated. In spite of the very small amount of 13MeM ingested, compared to the mass of adipose tissue, 13MeM:palmitate showed an increase at the three sites, with the largest one at the waist site (0·0110 and 0·0203 before and after 13MeM 14 d ingestion, respectively).

The use of a structurally-labelled fatty acid which is not significantly interconverted to other fatty acids should provide quantitative data on adipose tissue turnover in man at various subcutaneous sites. In addition it may provide a direct comparison of certain aspects of adipose tissue metabolism both in vivo and in vitro from the same biopsy material (Ashwell *et al.* 1977).

Ashwell, M., Durrant, M., Stalley, S. & Garrow, J. S. (1977). *Proc. Nutr. Soc.* **36**. (In the Press.)

Campbell, R. G. & Hashim, S. A. (1972). *Proc. Soc. exp. Biol. Med.*, **141**, 652.

Changes in blood metabolites following the acute ingestion of various amounts of glucose, fructose, sucrose and sorbitol. By I. MACDONALD and ANNE KEYSER, *Physiology Department, Guy's Hospital Medical School, London SE1 9RT*

Varying the amount of glucose given in a tolerance test has been found to have little effect on the blood glucose levels (Jourdan, 1972). The aim of this experiment was to carry out similar investigations using varying doses of sucrose, fructose and sorbitol as well as glucose, and to study various metabolites in the blood in addition to glucose.

After a 12 h fast, nine healthy male students were given, in random order, 0·25, 0·5, 0·75 or 1·0 g (except sorbitol) glucose, sucrose, fructose or sorbitol/kg body-weight (diluted with 4 ml water/kg body-weight). Venepuncture was done at 0, 15, 30, 60 and 90 min after ingestion of the solutions. Serum glucose, insulin, fructose, triglyceride, glycerol, uric acid, lactate and pyruvate were assayed. The results show that after the glucose tolerance test, apart from the expected increase in glucose and insulin, there was a significant fall in the concentration of triglyceride, glycerol and lactate. The sucrose meals were followed by an increase in glucose, insulin and fructose as found in previous work, together with a decrease in triglyceride and glycerol levels. There was also a significant rise in uric acid and pyruvate concentrations, although this was not found after the 0·25 g/kg body-weight dose.

Serum insulin and fructose concentrations were increased after 0·25 and 0·75 g sorbitol/kg body-weight. Pyruvate was also seen to rise. In addition a fall in triglyceride and glycerol was observed with this carbohydrate. The findings following fructose ingestion have been previously reported (Macdonald & Pacy, 1976).

These results confirmed that the amount of glucose ingested did not affect the rise in serum glucose. However, the serum insulin levels were dose related to glucose but not to fructose, suggesting that little fructose was converted to glucose. The fall in serum triglyceride level was not related to the insulin concentration as has been suggested (Jones & Arby, 1965), but the rise in uric acid, lactate and pyruvate levels associated with fructose ingestion were confirmed (Woods & Alberti, 1972). Glucose ingestion was followed by a decrease in blood lactate levels.

Jones, D. P. & Arby, R. A. (1965). *Metabolism* **14**, 1287.
Jourdan, M. H. (1972). *Guy's Hosp. Rep.* **121**, 155.
Macdonald, I. & Pacy, D. (1976). *Proc. Nutr. Soc.* **35**, 69A–70A.
Woods, H. F. & Alberti, K. G. M. M. (1972). *Lancet* **ii**, 1354.

Effect of insulin on protein turnover in foetal lambs. By S. CHRYSTIE, JANE HORN, I. SLOAN, M. STERN, D. NOAKES and M. YOUNG, *Department of Gynaecology, St. Thomas's Hospital Medical School, London SE1 7EH and Department of Obstetric Surgery, Royal Veterinary College Field Station, North Mimms, Herts.*

The possible role of insulin as a foetal growth hormone was assessed by its effect on free amino acid pools and rates of protein turnover in foetal tissues. The synthesis rate of mixed tissue proteins in a variety of organs has been reported (Young *et al.* 1976) in lambs at 135 d gestation, using the method described by Garlick *et al.* (1973). [^{14}C]lysine was infused continuously for 6 h, and the ratios of specific activities of lysine in the protein (bound) and intracellular (precursor) pools (S_B:S_i) were used to calculate half-lives of tissue proteins. In four experiments where the foetal acid–base balance was satisfactory (pH 7·36±0·01 and pO_2 20± 3 mm Hg, mean±SEM), mean half-lives for liver, brain, skeletal muscle and heart were 18, 22, 26 and 28 h respectively. These were similar to those observed for the newborn lamb, and considerably lower than in the adult (Buttery *et al.* 1975).

Infusion of insulin, simultaneously with the labelled amino acid, at rates of 0·26 to 1·66 U/h per kg foetal weight reduced free acid concentrations in all tissues studied except brain. Free lysine fell by up to 90% in skeletal muscle, with smaller reductions in cardiac muscle (up to 70%) and in liver (up to 40%). These changes were interpreted as being the result of reduced protein catabolism. However, blood glucose concentrations were low at the end of the experimental period with insulin, and the use of amino acids as a source of energy is possible; this was suggested by a fall in the proportion of the total tissue counts associated with the free lysine. Nevertheless, S_i of lysine rose in skeletal muscle, heart and brain. No change was observed for S_B in any of the tissues studied, which suggests that insulin had no effect on protein synthesis. Protein turnover rates were, however, reduced significantly ($P<0·05$) in both skeletal muscle and cardiac muscle. It appears that insulin may exert a growth-promoting action by reducing the catabolic portion of the anabolic–catabolic equilibrium of protein synthesis.

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Garlick, P. J., Millward, D. J. & James, W. P. T. (1973). *Biochem. J.* **136**, 935.
Young, I. M., Chrystie, S. & Sloan, I. (1976). *Fifth European Congress of Perinatal Medicine*, p. 52.

A survey of food habits and attitudes to diet among pregnant women and mothers of young children. By N. RUCK* and R. PARISH*, *Department of Nursing Studies, Polytechnic of the South Bank, Borough Road, London SE1*

A survey was carried out to investigate the attitudes to food held by women and the extent and sources of their nutritional knowledge. Thirty women, fifteen of whom were pregnant and fifteen with at least one child under 5 years, were interviewed in their own homes. They were given open-ended questions on how they chose their food, where they had received nutritional advice, a short 'nutrition quiz' and some questions on social background and food budget.

Almost all the subjects said that they valued animal foods (meat, cheese, eggs and milk) most, and carbohydrate (bread, biscuits and cakes) least. Very few of the subjects were concerned about animal fats possibly causing heart disease, and less than half cut down their sugar intake to avoid tooth decay. Fruit and vegetables more than unrefined cereals were mentioned as a good source of fibre. These nutritional beliefs have a strikingly different emphasis from those of nutritionists, as reported recently (Brown *et al.* 1977).

For most subjects, cost was the most important factor influencing food choice. Many subjects said that if they were richer they would eat more meat. Convenience foods, especially frozen foods, were used more often by subjects in social classes I and II (Registrar-General's classification).

Most subjects were concerned to eat a good diet and had considerable nutritional knowledge as measured by the quiz on nutrients. The subjects with young children scored better in this quiz than the pregnant subjects. For all the women, most of their information came from informal sources, such as literature, family and friends, rather than from the National Health Service. However, subjects with children had usually received some advice from health visitors on infant feeding, while there was little food advice given to the pregnant women or to the mothers on their own diets. There were some indications that more advice given anti-natally could have been beneficial to such highly motivated groups.

This study was done as part of the Diploma in Health Education at the Polytechnic of the South Bank.

Brown, C. L., Brown, A. M. & Naismith, D. J. (1977). *Proc. Nutr. Soc.* 36, 96A.

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Anaemia in infancy. By D. BURMAN and D. STEVENS, *Bristol Royal Hospital for Sick Children, Bristol BS2 8BJ*

Haemoglobin levels in infants in the first 2 years of life have been used to study the effect of dietary supplements of haematinics.

Normal-birth-weight infants have a mean cord haemoglobin of 17·05 g/100 ml with a standard deviation of 1·87 (Burman, 1959). Despite this wide range, birth weight is the most important factor determining iron stores at birth (Burman, 1971). Changes in the distribution of body Fe, plasma ferritin (Siimes *et al.* 1974), serum Fe, Fe-binding capacity (Sturgeon, 1954) and transferrin saturation are shown. Fe supplementation (10 mg/d) makes no significant difference to haemoglobin throughout the first 2 years of life or to the incidence of infection. This is in contrast to the effect found 50 years ago (Burman, 1972). If Fe deficiency is found today, blood loss from gastrointestinal or urinary tracts should be suspected. Causes of gastrointestinal loss include coeliac disease, oesophageal varices, hiatus hernia, ulcerative colitis and Meckels diverticulum.

Low-birth-weight infants (below 2·5 kg) have a low cord haemoglobin compared with mature infants and a marked sex difference in the relationship between gestational age and haemoglobin. 'Small-for-dates' infants have a higher haemoglobin than those whose weight is appropriate for gestational age (Burman & Morris, 1974). During the first year of life Fe supplementation is associated with haemoglobin levels similar to normal-birth-weight infants. Folic acid supplementation (0·1 mg/d) increases haemoglobin significantly with a difference in the region of 0·5 g/100 ml. The clinical importance of this finding is doubtful as there is no effect upon weight gain.

Very-low-birth-weight infants (under 1·5 kg) were supplemented with vitamin E (15 mg twice daily) but this made no difference to haemoglobin. The rise in haemoglobin between 2 and 3 months of age in supplemented infants is not significantly less than that previously described as response to vitamin E.

Conclusions. (1) There is no evidence to suggest that dietary supplementation of normal-birth-weight infants with iron is beneficial. (2) The higher haemoglobin found in low-birth-weight infants supplemented with folic acid is of doubtful significance. (3) Very-low-birth-weight infants do not benefit by vitamin E therapy.

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A dietary survey of the aged in the Southern Nigerian populations.

By D. O. NNANYELUGO, U. O. AKPANYUNG and L. O. KUBIANGHA, *Department of Food and Home Sciences, University of Nigeria, Nnukka, Nigeria.*

A survey was conducted in the mainland areas of the Cross River State between 4 December 1976 and 8 March 1977. A total of 307 elderly subjects consisting of 173 men and 134 women were investigated. The ages ranged from 60 to 102 years. From this number, a detailed weighed individual intake survey was carried out on 38 males and 26 females. Their average nutrient intake was calculated using food composition tables and compared with the FAO requirements. Information about foods eaten the previous day, source of foodstuff and income, method of food preparation and background details of the elderly were also collected. The weights of those who took part in the individual food intake survey as well as any clinical signs present were recorded.

The results of the survey showed that starchy foods and fruits formed the greatest source of the essential nutrients. The majority of the aged did not meet their minimum requirements for riboflavin, thiamine, niacin. There was low dietary intake of iron for women, but most of the males and females had adequate intakes for vitamins A and C and calcium and marginal intake for energy. This was due to a high consumption of vegetable oil, fruits and starchy foods; however, two of the aged were blind with low intake of vitamin A. The consumption of protein was satisfactory when compared with FAO requirements (FAO, 1973). The protein intake averaged 0·81 g (for men) and 0·93 g (for women) per kg body-weight. The significance of these findings as the proportion of the aged increases will be discussed.

FAO/WHO (1973). *Wld Hlth Org. tech. Rep. Ser.* no. 522.

Estimation of nitrogen metabolism in man using ^{15}N-labelled yeast protein. By D. HALLIDAY and W. W. C. READ (Introduced by J. S. GARROW)
Clinical Research Centre, Watford Road, Harrow, Middx.

We have developed a simple technique by which a single estimation of ^{15}N, in addition to N balance results, will provide values for utilization of dietary N, extent of re-utilization of catabolic N, and the rate of synthesis and catabolism of all N compounds in the body derived directly from amino acids.

5 mg ^{15}N as yeast protein is given by mouth with breakfast, and a portion of the following 72 h urine collection analysed mass spectrometrically for its ^{15}N content. ^{15}N-labelled yeast protein is used in preference to [^{15}N]glycine, since there is evidence that all amino acids are not treated by the body in an identical fashion, and that these differences may be accentuated in abnormal nutritional or metabolic states.

The parameters of N metabolism were calculated from the derived equations:

(1) urinary N from diet $= \frac{Ie^*}{d^*}$; (2) urinary N from catabolism $= E_t - \frac{Ie^*}{d^*}$

(3) total synthesis, N, $= \frac{E_t e^* - E_t}{d^*}$

(4) total catabolic N $= \frac{E_t d^* - I}{e^*}$

where e^* is cumulative excretion of ^{15}N at time t, d^* is ^{15}N administered, I is dietary N intake, E_t is total urinary N.

This method has been applied to two groups of obese female subjects. The first group consisted of three women maintained for 6 weeks on a constant diet (3·4 MJ; 42 g protein). The protein provided a slight excess of requirements regarding both total protein and essential amino acids. ^{15}N was administered on three occasions to each subject at 14 d intervals. All subjects were in negative N balance during the first two periods of the study, but became positive in the third. Both synthesis and catabolism fell between the first and second period (mean synthesis rate 21·3 to 16·4 g N/d; catabolism 21·9 to 17·1 g N/d) in all three subjects, but there was a tendency for both synthesis and catabolism to return to the initial values during the third. Values derived by the described method were consistently lower than those calculated from excreted ammonia- or urea-^{15}N levels. The second group of six subjects were provided with 800 kJ/d either as egg-white protein or cornflour which were switched after 7 d. ^{15}N administration was 72 h before the conclusion of each dietary period. The order of the diet presentation did not appear to effect the rate of synthesis or catabolism during each dietary regimen. The results obtained were somewhat ambiguous, but the change of diet appeared to rapidly effect dietary N retention and catabolism. Results obtained from the technique presented are compared with those obtained from the same study based on ammonia or urea as metabolic end-products.

Free amino-nitrogen levels across the hindquarters of sheep. By MARGARET I. CHALMERS, I. GRANT and F. WHITE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

We have measured the net change of free amino-N by arteriovenous difference across the hindquarters of sheep. Chronic intravascular catheters were placed in the aorta (A) and in the posterior vena cava (V) at a point posterior to the entry of the posterior renal vein: the catheters were positioned under X-ray screening. In each experiment blood samples were drawn from A and V every 30 min over a period of hours, packed cell volume (PCV) and free amino-N in whole blood and plasma were determined in all samples. The rations fed included dried grass cubes, mixtures of dried grass cubes with cereals or hay, barley cubes, cereals with hay and with added supplements of insoluble proteins (fish meal or formaldehyde-treated casein): the N intake ranged from 8 to 21 g/d. In 7 experiments casein was put directly into the abomasum to produce a rapid rise in the concentration of free amino-N in A. In all, 21 experiments have been done and 9 sheep used.

The concentration of free amino-N in V blood was higher than that in A suggesting a constant release of amino acids into the blood draining into V. The difference (V−), corrected against PCV for the movement of water, was positive in whole blood, the average difference in the 21 experiments being +0·35 to +4·40 mg N/l of input blood. In 5 experiments the net change of free amino-N in plasma was greater than that in whole blood and in 16 experiments it was less. No correlation was seen between the magnitude of the apparent release of free amino-N and N intake, ration fed or time after surgery.

In 188 paired samples of V and A blood there was no difference in the mean PCV. There was a highly significant increase in the mean concentrations of free amino-N in both plasma and cells of V blood compared with that in A blood. This supports the hypothesis that the blood cells are involved in the transport of amino acids and in the net change of amino acids across tissue.

We question whether by determining only free amino-N (a monitor of free amino acids) we have sampled the correct input pool in blood of the amino acids used for synthesis of tissue protein.

Availability of methionine and lysine in sorghum grain in relation to the tannin content. By J. E. FORD, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

In some food grains, part of the protein may be intrinsically of low digestibility, or become indigestible during processing through interaction with polyphenols which are widely present in higher plants. There is need therefore for caution in the interpretation of amino acid analyses in terms of nutritional quality.

Grain of ten varieties of sorghum was obtained, representing high- and low-tannin lines. Their content of total and available methionine, and the relative nutritional value (RNV) of the protein, was measured microbiologically with *Streptococcus zymogenes* as described by Ford (1962), except that the test samples were milled to pass an 80 mesh sieve and predigested with Pronase instead of papain. 'Reactive lysine' was determined by the differential dye-binding method of Hurrell & Carpenter (1975), and tannin content by the modified vanillin method (Maxon & Rooney, 1972). The results were assessed in relation to chick bioassay values obtained for the same materials by Nelson *et al.* (1975).

The chick values for 'average amino acid availability' ranged from 66 to 94% and were correlated (r −0·82; $P<0·01$) with tannin content in the samples (Nelson *et al.* 1975). The chick assay procedure employed has been reported as giving unduly high results (Elwell & Soares, 1974) and certainly a wider spread of values was obtained in the microbiological tests. Available methionine values ranged from 0·52 to 1·54 g/16 g N (33 to 100%) and were highly correlated with tannin content (r −0·98; $P<0·001$), as were the RNV values (r −0·97; $P<0·001$). Total methionine values varied little between samples (range 1·64 to 1·81 g/16 g N) and were not related to tannin content. The same was true for the reactive lysine values, which for these materials were clearly a fair measure of the total lysine present; they gave no inkling of the differences in the digestibility of lysine that were so evident in the chick assay results.

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Influence of polyethylene glycol and related compounds on the nutritional availability of methionine in a high-tannin sorghum and in field beans. By J. E. FORD, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Polyethylene glycol (PEG 4000) was examined for its influence on the nutritional availability of methionine in sorghum grain containing 3·2% tannin as measured by the modified vanillin method (Maxson & Rooney, 1972). The grain was milled to pass an 80 mesh sieve and samples weighed out containing 12·5 mg N. To each was added 10 ml 2% (w/v) solution of sodium β-glycerophosphate, and 1 ml water or aqueous solution containing 1, 5, 25 or 125 mg PEG 4000. The mixture was adjusted to pH 8·0 and 1 ml 1% (w/v) solution of Pronase added. The whole was then incubated 3 h at 48° in a shaker-incubator and assayed for available methionine and relative nutritional value (RNV) with *Streptococcus zymogenes* (Ford, 1962). Results were as follows:

PEG 4000		Methionine (g/16 g N)			RNV
Added (mg)	mg/g test protein	Total	Available	Available / total	(Casein = 100)
0	0	1·51	0·69	0·457	32
1	12·8		0·84	0·556	55
5	64		1·30	0·861	74
25	320		1·40	0·927	84
125	1600		1·44	0·954	85

In further tests PEG 4000 was compared with polyvinylpyrollidone (PVP), polyoxyethylene sorbitan oleate (Tween 80) and 'Lissapol NDB' (a non-ionic detergent, containing PEG with substituent phenyl groups), all at the 100 mg level (dry weight). All increased the measured available methionine in the sorghum more than twofold, but PVP gave marginally the greatest increase.

In field beans the seed-coat of coloured-flowered varieties was rich in tannin, whose presence was reflected in low values for available methionine in the testa which were increased twofold by inclusion of PVP in the assay. The effect of the tannin was less pervasive than in sorghum and was largely confined to the testa. Thus the potential for improvement in the digestibility of the protein of the whole grain through the use of PVP was comparatively small, about 10%.

Ford, J. E. (1962). *Br. J. Nutr.* **16**, 409.
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Influence of polyethylene glycol on digestibility of the protein in high-tannin sorghum in rats and chicks. By J. E. FORD and D. HEWITT, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Polyethylene glycol (PEG 4000) enhanced the nutritional quality of a high-tannin sorghum, as judged by microbiological tests (preceding communication). Its effect was examined with rats and chicks, using this same sorghum to supply 80 g crude protein/kg in the test diets.

In the rat tests, protein digestibility (D), biological value (BV) and net protein utilization (NPU) were determined by the balance method of Henry & Toothill (1962), modified in that each diet was given to three 4-week-old Sprague Dawley rats for a single period of 7 d. In the chick tests D was measured by the 'ileal analysis' method of Varnish & Carpenter (1975). Four 6-week-old RIR×LS chicks received each diet and ileal contents from pairs of birds were combined, giving two 'pooled' samples for each diet. Results were as follows:

PEG 4000	Chick test	Rat test		
(g/g crude protein)	D	D	BV	NPU
0	0·44	0·53	0·77	0·42
0·1	0·90	0·92	0·59	0·54
1·0	0·94	0·99	0·58	0·57
LSD (P=0·05)	0·10	0·07	0·11	0·11

With both test species PEG 4000 substantially increased protein digestibility. The high BV obtained in absence of PEG was undoubtedly spurious. Henry & Kon (1957) similarly obtained a markedly higher BV for deteriorated milk powder at 4 than at 8% level of protein intake and concluded that at low test levels of protein deficient in an essential amino acid, unduly high BV results are obtained.

In selecting for low tannin content in sorghum the plant breeder tends to lose several important agronomic advantages of the high-tannin lines (Harris, 1969). The use of PEG or related compounds in the formulation of sorghum diets might prove an economical alternative means for improving the efficiency of utilization of the high-tannin varieties.

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Duodenal glucose infusion and hepatic enzyme activities in sheep.

By E. F. UNSWORTH and J. PEARCE, *Department of Agricultural and Food Chemistry, The Queen's University of Belfast and Department of Agriculture, Northern Ireland*

It has been shown that concentrate feeding as compared with roughage feeding results in higher specific activities of hepatic carbohydrate metabolizing enzymes in sheep and cattle (Pearce & Unsworth, 1976*a*,*b*). These results suggest that a greater amount of glucose enters the duodenum of concentrate-fed ruminants than in animals on roughage diets. The present work was done to examine the effects of duodenal glucose infusions on some enzymes of hepatic carbohydrate metabolism.

Two groups of three cross-bred 2-year-old ewes, each fitted with simple T-piece cannulas in the proximal duodenum, were maintained on a grass-nut diet. After 2 weeks on this diet each animal in one of the groups received 264 ml glucose solution (640 g glucose/l)/d via the duodenal cannula for 10 d before liver biopsy. Liver biopsies were performed on the 6 sheep and liver cell-free extracts prepared as previously described (Pearce & Unsworth, 1976*a*) except that the extracts were made in 0·1 M-potassium phosphate buffer, pH 7·0, containing 7 mM-2-mercaptoethanol. The activities of some enzymes were determined in these extracts using methods previously outlined (Pearce & Unsworth, 1976*a*). Although there was no significant effect of glucose infusion on blood glucose content, analysis of variance showed that the specific activities of the pentose phosphate pathway enzymes, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, were higher in extracts from sheep receiving glucose. Similarly the specific activities of the glycolytic enzymes, phosphofructokinase, fructose diphosphate aldolase and pyruvate kinase, were elevated in liver extracts of sheep which received the glucose infusions.

Table 1. *The effects of duodenal glucose infusion on the specific activities (nmol substrate metabolized/min per mg protein in the extract) of some enzymes*

	Control	Glucose-infused	SEM (4 df)	(F test)
Glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49)	10·38	14·76	1·75	NS
6-Phosphogluconate dehydrogenase (*EC* 1.1.1.43)	102·01	161·48	8·30	$P<0·01$
Pyruvate kinase (*EC* 2.7.1.40)	165·36	242·86	15·02	$P<0·5$
Fructose diphosphate aldolase (*EC* 4.1.2.13)	91·92	116·92	20·40	NS
Phosphofructokinase (*EC* 2.7.1.11)	33·82	58·26	5·20	$P<0·5$

The results show that the infusion of glucose into the duodenum results in increases in the specific activities of some hepatic enzymes of glucose dissimilation.

Pearce, J. & Unsworth, E. F. (1976*a*). *Br. J. Nutr.* **35**, 407.
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Effect of different volatile fatty acid proportions on nitrogen balance in lambs fed entirely by ruminal and abomasal infusions. By E. R. ØRSKOV and D. A. GRUBB, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The maintenance of functional ruminants by intra gastric feeding alone has been reported by Tao & Asplund (1975) who gave mature wethers sufficient nutrients for energy maintenance. In the past 3 years we have developed this technique for the study of growth and energy utilization in lambs sustained for several months by infusion with almost normal rates of gain and normal health. This technique is suitable for the study of utilization of different volatile fatty acids (VFA) for ruminants, which has been the centre of considerable controversy in recent years (see Thomas, 1975).

Four lambs between 20 and 30 kg live weight (W) were given VFA and required macrominerals with buffer solution to maintain rumen pH between 6 and 7 via a rumen cannula. Casein and vitamins were given via an abomasal catheter, through which trace minerals were injected daily.

The level of casein infusion was 9·5 g protein/kg $W^{0.75}$ per d, calculated to be in excess of growth requirement. The total gross energy infused amounted to 940 kJ/kg $W^{0.75}$ per d. The live weight gain of the lambs ranged from 150 to 200 g/d during the experiment. Dried grass was also given, but with the high level of infusion little was consumed. A 4×4 Latin square design with 14 d periods was used to test four different proportions of VFA (see table).

The result of the infusion on urinary and total N excretions and N balance are given in table:

Proportion of volatile fatty acids			N intake (g/d)		N excretion (g/d)		N balance (g/d)
Acetic	Propionic	Butyric	Casein	Total	Urine	Total	
450	450	100	16·5	16·5	10·3	10·3	6·2
550	350	100	16·9	17·2	12·3	12·6	4·6
650	250	100	16·4	17·0	11·4	12·7	5·0
750	150	100	16·5	17·5	11·7	12·6	4·8
SE of treatment means			0·2	0·2	0·6	0·6	0·7

(Each value is the mean of 4 observations)

The small differences between casein and total N intake and urinary and total N excretion are due to the small amount of dried grass consumed and the consequent occasional faecal N excretion.

The results suggested that there was no detectable difference in N retention as a result of quite widely varying VFA proportions. The highest propionate proportion gave the lowest urinary excretion but the difference was not significant and there appeared to be no consistent linear or curvilinear trend in N utilization related to different proportions of VFA.

Tao, R. C. & Asplund, J. M. (1975). *J. Anim. Sci.* 41, 1653.
Thomas, P. C. (1975). *Wld Rev. Anim. Prod.* 11, 33.

The effect of alkali and urea on ground and pelleted all-straw diets for sheep. By E. L. MILLER, I. L. JOHNSON, M. C. E. BRIGGS and R. G. KEMPSEY, *Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX*

The voluntary intake and digestiblity of straw are low and result in inadequate energy and protein intake to meet the needs for maintenance of the ruminant. This experiment investigated whether alkali treatment and urea supplementation improved straw sufficiently to provide for maintenance. Straw was processed in a pilot-scale plant either by pelleting ground barley straw through a 10 mm die to produce untreated straw (UTS) or by spraying with NaOH (400 g/l) before pelleting to give 50 g NaOH/kg alkali-treated straw (ATS). Four treatments consisted either of UTS pellets sprayed with (1) water (2·5 l/50 kg), (2) urea solution (1250 g in 2·5 l/50 kg) or ATS pellets sprayed with (3) water (3·75 l/50 kg), (4) urea solution (1250 g in 3·75 l/50 kg).

Each of eight wethers, weighing 30 kg and fitted with a rumen cannula, were given the four pelleted diets *ad lib.*, plus 40 g/d of a mixture of minerals and vitamins with Molassine Meal (Molassine Meal Co. Ltd, London), according to sequences that gave an element of balance over periods. A period consisted of 17 d following 4 d for changeover between diets. Dry matter (DM) intake and digestibility were determined and rumen samples were obtained on days 4–17, 12–17 and 12–14 respectively.

Diet	UTS	UTS+ urea	ATS	ATS+ urea	SEM	Effects
Rumen pH	7·31	7·21	7·50	7·23	0·030	U***, A**, UxA*
Rumen ammonia (mmol/l)	0·64	6·85	0·44	6·51	0·651	U***
DM intake (g/d)	568	870	429	1143	71·8	U***, UxA*
DM digestibility	0·30	0·46	0·51	0·64	0·035	U***, A***
Digestible DM intake (g/d)	158	385	197	734	57·1	U***, A**, UxA*

U effect of urea; A effect of alkali; U×A interaction. $^{*}P<0·05$; $^{**}P<0·01$; $^{***}P<0·001$.

Both alkali treatment and urea increased DM digestibility and their effects were additive. Alkali treatment only increased DM or digestible DM intake in the presence of urea. In the absence of urea rumen ammonia concentrations were less than the suggested requirement for optimal microbial growth (Roffler *et al.* 1976) which may explain the responses to urea supplementation. Rumen pH values were generally high; alkali treatment only increased pH in the absence of urea.

Therefore, at least for a short period, ATS supplemented with urea, minerals and vitamins could be the sole feed for maintenance of sheep without causing obvious metabolic upset.

Roffler, R. E., Schwab, C. G. & Satter, L. D. (1976). *J. Dairy Sci.* 59, 80.

A concentrated solution containing urea, phosphorus, calcium and sodium for supplementation of oat straw-based diets given to beef cows. By R. G. HEMINGWAY, J. J. PARKINS and G. FISHWICK, *Glasgow University Veterinary School, Bearsden, Glasgow*

A range of acidic, soluble supplements (LS) was developed containing (g/kg) up to 1200 crude protein (CP, as urea), 30 calcium, 15 phosphorus, 20 sodium and 100 molasses plus trace elements with specific gravity 1·3. LS is suitable for incorporation in rolled cereals, sugar beet pulp, straws and hays, maize silage and drinking water to supply about 0·25 kg LS/d to 500 kg cows.

Eighteen pregnant cows in a Latin square design (3 week periods) were given either 60 g urea/d in 2 kg barley in one (U) or four one-quarter (4U) feeds/d plus straw *ad lib.*, or 2 kg barley plus straw containing 35 g LS/kg dry matter (DM) (injected at 40 different sites in each 20 g bale) *ad lib.* to provide about the same total N intake. The voluntary straw DM intakes were 6·30 (U), 6·39 (4U) and 6·57 (LS) kg/d. The whole diet dry organic matter digestibility percentages (DOMD %s) were 52·4 (U), 53·7 (4U) and 59·0 (LS)*** and for the straw alone (if the barley was 86% digestible) were 43·0 (U), 44·8 (4U) and 51·8 (LS)***. Blood and rumen liquor ammonia concentrations were similar over a 24 h period other than for an initial rise 2 h after U was given.

In a trial over the first 6 weeks of lactation one group of 10 cows (410 kg) was given straw *ad lib.* plus 3 kg cubed barley containing 90 g urea. The other group was given 3 kg cubed barley and LS-treated straw *ad lib.* to provide about the same total urea/d. Voluntary consumption (kg DM/d) of the straws and whole diet DOMD %s were 6·03 and 42·0 (untreated) and 6·35 and 48·3 (LS treated). Cow live-weight changes and calf live-weight gains (0·75 kg/d) were comparable for both groups.

In two subsequent trials, 500 kg beef cows were given straw and barley in fixed amounts to meet their metabolizable energy (ME) requirements for the last 8–9 weeks of pregnancy and the first 6 weeks of lactation. In the first trial (10 cows/group) straw:barley DM ratios were 3·2:1 (pregnancy) and 2·8:1 (lactation), and 0·25 kg CP as LS was given either in the baled straw or in the rolled barley. The whole diets DOMD %s in both pregnancy and lactation (mean 56·4), cow live-weight changes and calf live-weight gains (0·57 kg/d) were similar for both groups. In the second trial (7 cows/group) the straw:barley DM ratios were 2·4:1 (pregnancy) and 2·2:1 (lactation). Protein supplementation was either nil or 0·25 kg digestible CP/d given as either groundnut (G) or LS included in cubed barley. The whole diet DOMD %s in pregnancy (mean, 2 determinations/cow) were 52·4 (nil), 52·7 (G) and 56·0 (LS)*, but with no difference in lactation. In pregnancy cows given G or LS gained more live-weight ($P<0·05$) but all groups had the same weight loss in lactation. Calf live-weight gains were 0·65 (nil), 0·76 (G) and 0·79 (LS) kg/d (LSD at $P=0·05$, 0·14 kg/d).

How linoleic acid (C18:2) affects the response of guinea pigs to basic protein. By C. J. MEADE, J. MERTIN, JINAN SHEENA and RUTH HUNT (Introduced by J. S. GARROW), *Transplantation Biology Section, Division of Surgical Sciences, Clinical Research Centre, Watford Road, Harrow, Middlesex*

Basic protein (BP), a protein extracted from nervous tissue, will, when injected in complete Freund's adjuvant (CFA), produce an autoimmune disease characterized histologically by cuffs of mononuclear cells around the blood vessels of brain and spinal cord, and clinically by paresis, paraplegia and loss of bladder control. There is a lively controversy whether or not this disease, experimental allergic encephalomyelitis (EAE), is a useful animal model for multiple sclerosis. We report here that linoleic acid (C18:2) supplementation of the diet lowers the incidence of clinical signs in guinea pigs immunized to produce EAE.

Following injection of sufficient BP in CFA to produce only a mild encephalomyelitis (i.e. a dose of 10 μg of our preparation) total incidence of clinical signs of EAE was: unsupplemented animals 13/24 (54%), animals fed a normal diet supplemented with 0·5 ml C18:2/d 4/20 (20%), animals fed a diet supplemented with 0·5 ml water 12/23 (52%), animals fed 0·5 ml liquid paraffin 11/24 (46%). Frequency of and severity of perivascular lesions in the spinal cord and a crude, but objective criterion of disease, weight loss, were also reduced. C18:1 also had some encephalomyelitis suppressing activity.

It was found necessary to provide all animals with a supplement of vitamins A, D, E and K to compensate for the effect of the high fat content of some of our diets on intake of these vitamins. Provided this was done, animals given CFA without BP, and fed according to our protocol, appeared healthy.

The proportion of C18:2 in the serum was raised by a single oral dose of 0·5 ml C18:2. This increased ratio was maintained by continued feeding, but fell to normal levels 7 d after C18:2 supplementation was discontinued. C18:2 fed just before and during the normal time of appearance of signs of EAE (for up to 14 d) was effective, but if feeding was stopped about a week before this time, there was less suppression of disease.

C18:2 feeding for 14 d raised the level of C18:2 in CFA-stimulated lymph nodes markedly, but the pattern of fatty acids in the brain was hardly affected. Lymph node cells from animals given C18:2 (but only just before and during the time of appearance of clinical signs) had diminished ability to react to BP in vitro, as assayed using the gold uptake assay (Meade *et al.* 1974).

Meade, C. J., Lachman, P. J. & Brenner, S. (1974). *Immunology* 27, 227.

An automated optical emission spectrometer for ^{15}N analysis. By J. D. S. GOULDEN and D. N. SALTER, *National Institute for Research in Dairying, Shinfield, Reading GS2 9AT*

The rate of analysis of ^{15}N by emission spectrometry is inevitably slow because the preparation of pure nitrogen gas from the samples and filling of the emission tubes is a lengthy and tedious operation. We have now developed an automated emission spectrometer which eliminates this stage and carries out the preparation of N_2 from solutions of NH_4Cl automatically. A novel method of N generation is used in which the NH_4Cl sample (about 5 μl solution containing about 5 μg N) is injected in a stream of purified helium into a soda-lime reactor at 590°. Released ammonia flows through a catalyst tube where N is generated and is separated from the hydrogen by a gas chromatographic column which also retains the water. The N then flows through a pressure restrictor into a Spectrosil discharge tube in a microwave cavity. A specially constructed dual-wavelength monochromator is used to analyse the emitted radiation and enables the intensities of the $^{14}N^{14}N$ (297·7 nm) and $^{15}N^{14}N$ (298·3 nm) bands to be measured simultaneously by two photomultipliers. Signals proportional to the peak intensities are amplified and fed through phase-sensitive detectors into a ratiometer whose output is fed to a digital voltmeter and printed out in terms of ^{15}N abundance. A peak detector actuates the printer and records the total N present in each sample. Since the instrument is temperature sensitive, it must be maintained at a constant temperature of $23 \pm 0{\cdot}2°$.

Calibration is achieved by running NH_4Cl standards of known ^{15}N abundance and samples are analysed at the rate of approximately 1/min. Allowing time for stabilization and for running standards, about 250 unknown samples can be analysed in a normal working day. Carry-over between samples is very small and may be eliminated by running duplicates.

The precision of the ^{15}N-autoanalyser was compared with that of the Statron NOI-4 spectrometer. Standard deviations of replicate measurements of natural abundance (0·37 atoms percent ^{15}N) by the automatic analyser and the Statron were, respectively, 0·01 and 0·02. For enriched standards containing 1·44 and 2·54 atoms percent ^{15}N the standard deviations were, respectively, 0·01 and 0·01 for the automatic analyser, and 0·02 and 0·03 for the Statron. Determinations with the automatic analyser of ^{15}N in biological samples of widely varying origin were shown generally to be accurate to ±0·01 atoms percent.

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A simple unit for extracting protein in bulk from leaves. By J. B. BUTLER and N. W. PIRIE, *Rothamsted Experimental Station, Harpenden, Herts*

Most of the leaf protein (LP) used in human feeding trials was made from crops put successively through a chaff-cutter, a pulper and a belt-press. This elaborate process is simply a scaled-up version of the technique that is essential in agronomic work (Pirie, 1978). Now that the idea of using LP is gaining acceptance, there is an incentive to simplify the process of extraction.

A simplified screw expeller, modified so as to rub the leaf adequately before pressure is applied, has already been described (Pirie, 1977). That unit expressed juice in one operation but would not work satisfactorily on material that had not been through a chaff-cutter. By introducing an epicyclic gear, which ensures that the section of the expeller in which there is pressure always overtakes the section in which there is rubbing, and by introducing a mechanism that automatically stops the inflow of crop when there is an incipient overload, we have made a prototype one-piece extractor. This can conveniently be used to produce quantities of juice containing 1–10 kg LP from crops without chaff-cutting.

The unit demonstrated is a little more elaborate than is necessary because it is designed to allow more adjustment than would be needed on a production model, it also allows power consumption to be measured. It would, nevertheless, be much cheaper than existing equipment: it is also more economical. It uses about 300 W when processing 100 kg (fresh weight) crop/h. Larger units, working on the same principle could be made. There is more fibre in juice made in any unit based on the principle of the screw-expeller than in the pulper plus belt-press combination. Part of this fibre will contaminate the LP, but probably not to a harmful extent.

Pirie, N. W. (1977). *Expl Agric.* **13**, 113.
Pirie, N. W. (1978). *Leaf Protein and Other Aspects of Fodder Fractionation.* London: Cambridge University Press. (In the Press.)