The Two Hundred and Eighty-fifth Scientific Meeting of the Nutrition Society was held in the Faculty of Letters Lecture Theatre, Whiteknights Park, University of Reading, on Friday 26 September 1975, at 09.00 hours, when the following papers were read:

Carbohydrases in the small intestine mucosa of the pig. By D. E. KIDDER and M. J. MANNERS, *Departments of Veterinary Medicine and Animal Husbandry, University of Bristol, Langford House, Langford, Bristol BS18 7DU*

Samples of mucosa were taken at 5% intervals along the length of the small intestines of pigs of different ages from 3 weeks to 4 years. The levels of lactase (β-galactosidase, *EC* 3.2.1.23), trehalase (*EC* 3.2.1.28), sucrase (glucosidosucrase, *EC* 3.2.1.20), isomaltase (*EC* 3.2.1.10), maltase II and maltase III (both α-glucosidases, *EC* 3.2.1.20) were then measured in mucosal homogenates made from these samples.

The 3-week-old pigs differed from the older pigs in their enzyme distribution patterns. In particular, lactase was high in all but the distal 10–15% of the small intestine. In the older pigs (12 weeks–4 years) the pattern of distribution did not change appreciably with age, but the level did, with some decrease in lactase with age and an increase up to 8 months of age in the other enzymes. Each enzyme had a characteristic distribution pattern along the small intestine. Lactase had a peak level at 10–15% along the small intestine and then decreased to zero about half-way along. Trehalase had a peak level at 10–20% along the small intestine and decreased gradually to zero at the distal end. Sucrase and isomaltase were highest over the region from 15 to 85% of the length and were very low at the proximal end. Maltase II was maximal from 10 to 65% of the length. Maltase III was distributed fairly uniformly but was slightly lower at the proximal and distal ends then elsewhere.

There was considerable variation between pigs of the same age in the level of any particular enzyme, but the levels of the sucrase and isomaltase varied together. The levels of maltase II and III also tended to vary together but were less closely correlated than the sucrase and isomaltase.

These results confirm the findings on distribution of lactase, sucrase and trehalase reported in smaller previous studies (Manners & Stevens, 1972; Stevens & Kidder, 1972), and show that the changes of enzyme level with age reported for lactase and sucrase in younger pigs (Bailey, Kitts & Wood, 1956; Walker, 1959; Manners & Stevens, 1972) continue well beyond the weaning period.

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Lactic acid utilization by the baby pig. By ANNE CHRISTIE and P. D. CRANWELL, *School of Agriculture, La Trobe University, Bundoora, Victoria* 3083, *Australia*

Pigs born and reared under conventional conditions are known to have large amounts of lactic acid in their stomach contents, whereas minimal amounts are present in the stomach contents of pigs born in a clean environment (Cranwell, Noakes & Hill, 1968). Since the gastric flora are capable of fermenting lactose to produce comparable amounts of L(+)- and D(−)-isomers of lactic acid (P. D. Cranwell, D. E. Noakes & K. J. Hill, unpublished results) and, at least in other species, the D(−)-isomer is not readily utilized by mammalian systems (Cori & Cori, 1929; Drury & Wicke, 1956; Dunlop & Hammond, 1965), the suckling pig could lose a significant proportion of dietary energy by fermentation of lactose to the D(−)-isomer.

In order to determine whether the suckling pig can utilize D(−)-lactic acid young pigs were given a milk diet containing racemic lactic acid (Whittakers, Cranwell & Johnston, 1974). It was found that a considerable increase in urinary lactic acid excretion was due mainly to the presence of the D(−)-isomer (65–80% of that excreted) but there was also an increase in the excretion of L(+)-lactic acid.

Further experiments, involving five pairs of 10–14-d-old litter-mate pigs, were done to study the uptake and metabolism of the two lactate isomers. Total radioactivity in blood (Laurencot & Hempstead, 1971) and carbon dioxide radioactivity in blood and expired air (Harlan, 1961; Leng & Leonard, 1965) were measured following injection of ^{14}C-labelled sodium L(+)- or D(−)-lactate into the duodenum of anaesthetized pigs. The results indicate that both isomers are absorbed rapidly and at a similar rate from the small intestine. However it appears that the L(+)-isomer is utilized more rapidly initially, but that there is virtually no quantitative difference in utilization of the two isomers over a period of 4 h. The quantity of lactate used in these experiments was very small (25–140 μg), and it remains to be determined whether both isomers are utilized to the same extent when larger quantities of lactate are absorbed from the gastrointestinal tract.

This work is supported by the Australian Pig Industry Research Committee.

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Gastric secretion in the young pig. By P. D. CRANWELL, *School of Agriculture, La Trobe University, Bundoora, Victoria* 3083, *Australia,* and D. A. TITCHEN, *Department of Veterinary Preclinical Sciences, University of Melbourne, Parkville, Victoria* 3052, *Australia*

Separated Heidenhain fundic pouches, lacking vagal innervation, were prepared in thirty-four piglets aged from less than 24 h to 24 d. Secretion of acid was detected within 24 h of birth in the suckled piglet and was continuous while piglets were allowed to feed from the sow. If instead of being fed naturally (normally at hourly intervals) the piglets were separated from their dam, the volume and later the hydrogen ion concentration of the secretion from the pouch was reduced and secretion virtually ceased if the period of separation was maintained for 2·5–6·0 h. When normal feeding was resumed following fasting, secretion increased relatively slowly and the H^+ concentration did not return to the prefasting level within 120 min. The explanation advanced for these secretory responses is that they occurred following secretion of gastrin. Such secretion may have been in response to entry of food into the stomach or to vagal stimulation of the pyloric antrum.

Although acid is secreted by suckled piglets from within a few hours of birth the output of acid (H^+ concentration × volume) from pouches gradually increased in the 3–4 weeks after birth; this resulted from an increase in volume of secretion. A second, more rapid period of increase in volume of secretion occurred after 3–4 weeks.

Secretion of proteolytic enzymes also showed a progressive increase after birth. In the first 1–2 weeks little or no proteolytic activity was detected. An increase in proteolytic activity in the 2nd–3rd week after birth was followed by a dramatic increase in the 3rd and 4th weeks. In contrast to the increase in secretion of acid, this increase in proteolytic activity resulted from increases in both the concentration of enzyme and the amount of gastric juice secreted.

Since the main increase in proteolytic activity appeared to occur when the piglets started eating solid food, an attempt was made to determine whether solid food stimulated this increased proteolytic activity. Secretion by piglets given access to solid food was compared with that of litter-mates not given such access. Greater concentrations of pepsin were present in secretions of animals receiving solid food.

These results suggest that hormonal influences may be important in the development of the secretory capacities of the stomach. Gastrin has been shown to influence the maintenance of the population of parietal cells in adult animals (Crean, Marshall & Rumsey, 1969). Whether it has similarly important actions on the development of peptic cells in the young piglet has still to be studied.

This work is supported by the Australian Pig Industry Research Committee.

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Preparation and utilization of isolated hepatocytes for the study of sheep liver metabolism. By R. ASH, K. R. F. ELLIOTT and C. I. POGSON, *Biological Laboratory, University of Kent, Canterbury, Kent CT2 7NJ*

Recent studies of ruminant liver metabolism have centred on the use of two experimental techniques, namely, the perfusion of the excised liver (Lindsay, Jarrett, Mangan & Linzell, 1975), and the preparation of animals with chronically catheterized portal–hepatic venous systems (Bergman & Wolff, 1971). These techniques, although having much to recommend them, are nevertheless expensive and prove difficult to initiate and perform routinely. This communication describes a new approach which is relatively inexpensive and also allows a large number of variables to be studied under controlled experimental conditions. The method involves the preparation and utilization of suspensions of enzymatically isolated, metabolically active, ovine hepatocytes.

Cell suspensions were prepared from portions of sheep liver removed immediately after slaughter. The tissue was cut into slices and a total of about 5 g was placed in each of three flasks containing oxygenated calcium-free buffer (plus 10 mM-propionate). Dispersion of the liver tissue was obtained by incubation for 1 h at 37° in the presence of collagenase and hyaluronidase. After incubation, the contents of the flasks were filtered and the filtrate sedimented at 40 g for 2 min. The supernatant fraction was removed, and the cells washed twice in propionate-free buffer containing Ca. After the final centrifugation the cells were resuspended in buffer and used immediately.

The viability of the cells was checked by monitoring the following: (1) appearance under light- and electron-microscopy; (2) ability to exclude the vital dye, Trypan Blue; (3) the ratio, glutamate dehydrogenase (*EC* 1.4.1.2): lactate dehydrogenase (*EC* 1.1.3.2) in the cells and in an homogenate of the corresponding whole liver; (4) metabolic activity. Glucose production from propionate (Table 1) was routinely used to test metabolic activity since it is dependent on the continuing integrity of both cytoplasmic and mitochondrial compartments.

Table 1. *Glucogenic capacity of isolated sheep liver hepatocytes, using various substrates (concentration 10 mM): rates are expressed as a percentage of the rate with propionate**

Substrate	Rate
Propionate	100
Fructose	102
Pyruvate	38
Lactate	29
Glycerol	28
Proline	24
None	17

*Absolute value for rate with propionate, 179 nmol/mg dry wt per h.

This work was supported by the Medical Research Council.

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Hepatic gluconeogenesis in foetal and suckling lambs. By P. M. J. SAVAN, MARJORIE K. JEACOCK and D. A. L. SHEPHERD, *Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ*

Hepatic gluconeogenesis is the main source of blood glucose in adult sheep (Leng, 1970). Although Ballard & Oliver (1965) have found that the incorporation of ^{14}C-labelled propionate and pyruvate into glucose and glycogen occurs in foetal lamb liver slices, the extent of glucose production by foetal sheep liver has not been assessed. Glucagon is known to increase gluconeogenesis in perfused livers of some species (Exton, Mallette, Jefferson, Wing, Friedmann, Miller & Park, 1970). The purpose of this study was to determine the rate of gluconeogenesis in perfused livers of foetal and suckling lambs in the presence of alanine or lactate or propionate. The effects of glucagon on this process was evaluated and the results (mean values with their standard errors; no. of perfusions in parentheses) were:

	Rate of gluconeogenesis (nmol glucose/g tissue per min) with:					
	Alanine*	Alanine + glucagon†	Lactate*	Lactate + glucagon	Propionate‡	Propionate + glucagon
Foetal lamb liver	−96 ±76(3)	309 ±148(3)	−112 (1)	145 (1)	−361 ±151(5)	343 ±165(5)
Suckling lamb liver	157 ±36(4)	374 ±49(4)	282 ±70(4)	458 ±93(4)	326 ±210(3)	534 ±163(3)

*Alanine or lactate was infused into the perfusion medium at an average rate of 0·93 μmol/g tissue per min.

†Glucagon was added to the perfusion medium to give an average initial concentration of $3·3 \times 10^{-7}$ mol/l.

‡Propionate was infused into the perfusion medium at an average rate of 1·78 μmol/g tissue per min.

Gluconeogenesis, as measured by changes in the total glucose and glycogen content of the liver and the glucose content of the perfusion medium, occurred only in the presence of glucagon in the foetuses. Since glucagon has been shown to be present in both the pancreas and blood of the foetal lamb (Alexander, Assan, Britton & Nixon, 1971) it would seem possible that hepatic gluconeogenesis could occur in the sheep foetus in utero. However, in suckling lambs gluconeogenesis occurred in the absence of glucagon, and glucose production could account for 43, 67 and 40% of the hepatic uptake of alanine, lactate and propionate respectively. Addition of glucagon increased the rate of gluconeogenesis from alanine and lactate, apparently by increasing the proportion of substrate taken up which was converted to glucose.

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Inhibition of the biohydrogenation of dietary C_{18} unsaturated fatty acids by rumen bacteria using some inhibitors of methanogenesis. By P. KEMP and D. J. LANDER, *Department of Biochemistry, ARC Institute of Animal Physiology, Babraham, Cambridge*

Both long-chain unsaturated fatty acids (Czerkawski, Blaxter & Wainman, 1966; Czerkawski, 1969) and halogenated hydrocarbons (Bauchop, 1967) have been shown to decrease methanogenesis by rumen organisms, and Lanigan (1972) has reported that halogenated methane analogues also increase the in vivo and in vitro reductive cleavage of pyrrolizidine alkaloids by rumen bacteria. These results suggested that there might be competition for hydrogen between methanogenesis, the biohydrogenation of unsaturated fatty acids, and the reductive cleavage of the alkaloids, but the results presented here suggest that this is probably not so.

A single dose of carbon tetrachloride (2 g), chloroform (2·5 g) or Amichloral (2·5 g) (a gift from Smith Kline and French Laboratories, Welwyn Garden City) mixed with 1 l rumen fluid, administered to adult fistulated Clun Forest wethers (fed 1 kg hay chaff and 100 g crushed oats daily), resulted in a marked decrease in the biohydrogenation of dietary C_{18} unsaturated fatty acids. CCl_4 produced the greatest effect and Amichloral the least, though its effect persisted longer. Normal patterns of biohydrogenation returned within 4–6 d.

In vitro the biohydrogenation of added linoleic and linolenic acids by mixed populations of rumen organisms in fresh, strained rumen fluid was also inhibited by the three compounds.

In experiments with two pure cultures of anaerobic bacteria, F2/6 (a Gram-positive coccus) and *Fusocillus babrahamensis* (nov. spec. NCIB no. 10838) (P. Kemp, R. W. White & D. J. Lander, unpublished results), both with biohydrogenation activity towards linolenic and linoleic acids, partial inhibition (10–20%) of the reduction of these acids was obtained with low levels (0·1 mg/ml) of CCl_4 and $CHCl_3$. The hydrogen concentration in the gas phase increased but there was no apparent inhibition of bacterial growth. At the high levels of CCl_4 and $CHCl_3$ (1–2 mg/ml) necessary to produce a large inhibition of reduction (40–90%), the growth of cultures was severely impaired. No methane was found in the gas phase with these two bacteria under normal conditions of growth. The two bacteria do not appear to be dependent on unsaturated fatty acids for growth nor is their growth inhibited by the addition of these acids to the growth medium.

The addition of sodium formate or vitamin B_{12} to the culture medium did not affect growth, biohydrogenation or the extent of inhibition by CCl_4, $CHCl_3$ and Amichloral.

It appears that only the reduction steps are affected since in in vitro experiments there is a large increase in conjugated acids, and conjugation is the first step in the biohydrogenation of both linoleic and linolenic acids (Dawson & Kemp, 1970).

The implications of these findings will be discussed.

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Absorption of magnesium in the stomach of the ruminant. By JANE P. HORN and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Absorption from the stomach may form an important part of total magnesium absorption by the ruminant (Grace, 1970) but the site of absorption within the stomach and factors influencing its extent are poorly understood.

Steers (26–57 weeks of age) with simple cannulas in the rumen and proximal duodenum received diets of: (A) flaked maize, (B) flaked maize–hay, 1:1, (C) dried grass–starch, 3:2, (D) dried grass, or were allowed to graze spring grass. Stall diets were given at 09.00 and 17.00 hours. A mixture containing 30–60 g $MgCl_2.6H_2O$, polyethylene glycol (PEG) and a particulate marker ($^{144}CeCl_3$ or Cr_2O_3) was added to the rumen at about 10.00 hours. Changes in Mg and marker concentrations in digesta, the former corrected for background values obtained when no Mg was infused, were studied. In some experiments the need to correct for background Mg was avoided by the use of $^{28}MgCl_2$.

No changes in Mg:PEG ratios in the reticulo-rumen attributable to Mg absorption from that organ were found. However, varying amounts of Mg appeared to be absorbed up to the proximal duodenum. This absorption was assessed by preparing graphs relating concentrations of Mg and markers in duodenal samples with time after addition, as shown in Fig. 1, and comparing areas under the curves.

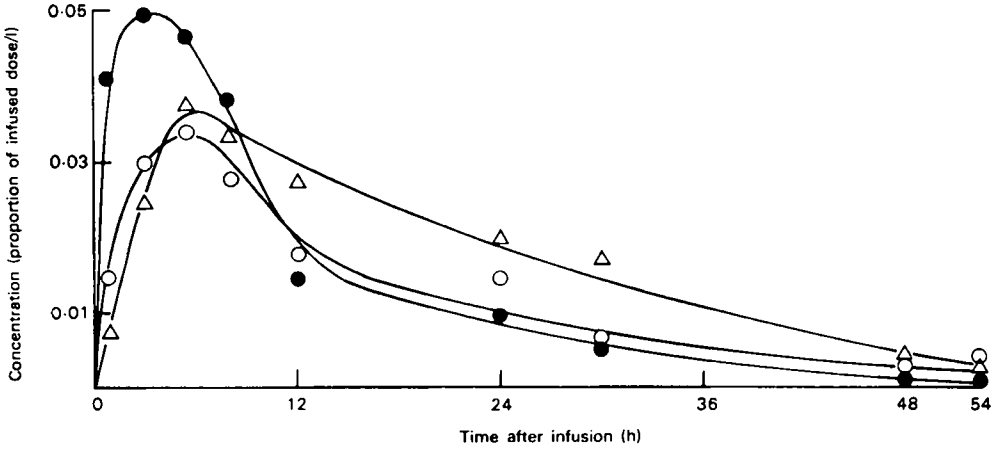

Fig. 1. Relative recoveries at the duodenum of magnesium (○), polyethylene glycol (●) and ^{144}Ce (△) after these substances were infused into the rumen of a calf given diet B (flaked maize–hay, 1:1).

For three calves given diets A, B, C or pasture, proportions of added Mg absorbed up to the proximal duodenum (mean±SE) were 0·24±0·04, 0·20±0·01, 0·11±0·05 and −0·05±0·09 respectively; average rumen pH values 2–5 h after feeding were 5·6, 6·6, 6·7 and 7·2 respectively. Infusion of 0·2 M-HCl (100 ml/h) into the rumens for 8 h after the calves received diet A caused a small decrease in rumen pH to 5·4 and a significant ($P<0·05$) increase in Mg absorption to 0·58±0·04. For two calves given diet D, similar experiments but with $^{28}MgCl_2$ addition showed the proportion of Mg absorbed to be 0·12±0·06 (rumen pH 6·7). This increased to 0·22±0·04 when 0·3 M-H_2SO_4 (500 ml/h) was infused into the rumen to give an average pH value of 6·1.

It appeared that although Mg absorption in the stomach occurred below the reticulo-rumen (probably in the omasum) its extent may have been related to rumen pH. It seems possible that the susceptibility of spring grazing animals to clinical hypomagnesaemia may be associated with poor absorption of Mg in the stomach partly as a result of a constant rather high pH in the rumen.

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The influence of sulphur compounds on the availability of lead to rats.

By J. QUARTERMAN, W. R. HUMPHRIES and J. N. MORRISON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The addition of sulphate to the food of sheep given lead improved growth, prevented anaemia and reduced the Pb content of bones and soft tissues (Morrison, Quarterman, Humphries & Mills, 1975). In the ruminant, sulphate can be metabolized to sulphide or sulphur amino acids. The effect of supplements of these compounds on the carcass and blood Pb contents was estimated in rats given Pb in the food.

Rats were given a semi-synthetic diet containing 200 g casein/kg (Williams & Mills, 1970) supplemented with Pb and S amino acids. Pb was estimated by atomic absorption spectrophotometry, using whole blood, after haemolysis with Triton-X (Kubasik, Volosin & Murray, 1972), and in the gut-free carcass.

A supplement of cystine and methionine did not affect growth or the accumulation of Pb in the carcass of rats but reduced the blood Pb concentration (Expt 1, Table 1). On the other hand supplementation with cystine alone increased the amount of Pb retained in the carcass but did not significantly affect the blood Pb concentration, whereas a methionine supplement increased growth, reduced the amount of Pb retained and greatly decreased the blood Pb concentration (Expt 2, Table 1). When rats weighing 43±0·5 g were given this diet without Pb but with or without supplementary methionine the body-weight gain in 4 weeks was not significantly affected (137±4 and 147±4 g body-weight gain respectively). In other experiments we have shown that when food intake and hence growth rate were reduced, the proportion of dietary Pb retained in the body was increased but no

Table 1. *Effects of supplements of cystine (5 g/kg diet) and methionine (6 g/kg diet) to the food of rats given 400 mg lead/kg diet as lead acetate for 3 weeks on the Pb content of blood and carcass*

(Mean values with their standard errors)

Addition to food	No. of rats	Final body-wt (g)	Carcass Pb (mg/kg)	Blood Pb (mg/l)
		Expt 1 (mean starting wt 45 g)		
Pb only	10	120±3	10·2±0·7	0·94±0·08
Pb+Cys+Met	10	130±5	10·7±0·6	0·33±0·03*
		Expt 2 (mean starting wt 30 g)		
Pb only	8	80±3	24·3±1·4	1·03±0·06
Pb+Cys	8	80±2	31·7±2·1*	0·81±0·14
Pb+Met	8	120±3	14·5±1·9*	0·09±0·03*

Cys, cystine; Met, methionine.
Values significantly different from those for group receiving Pb only: *$P<0.05$.

change was found in blood Pb concentration (Quarterman, Morrison & Humphries, unpublished results). In Expt 2 the decreased retention of Pb in the methionine-supplemented group may, therefore, be related to the greater growth rate of rats in this group, but the decreased blood Pb concentration associated with the methionine supplementation cannot be explained by differences in the rate of growth. When methionine was given intraperitoneally (at 250 mg/kg body-weight per d), neither growth nor Pb retention was affected but blood Pb concentration was again reduced. Thus methionine influences Pb metabolism after the Pb has been absorbed by the gut.

The addition of sodium sulphate (3 g S/kg diet) had no effect on carcass or blood Pb but when the Pb was given as lead sulphide (400 mg Pb/kg diet), carcass and blood contents were about half those found when Pb was given as the acetate.

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Selenium levels in human blood in New Zealand. By MARION F. ROBINSON, HEATHER REA and R. D. H. STEWART, *Department of Nutrition and Department of Medicine, University of Otago, Dunedin, New Zealand*

The concentration of selenium in whole blood of New Zealand residents (mean and SE 0·07±0·013 μg Se/ml) is lower than values reported from most other countries (Griffiths & Thomson, 1974; Watkinson, 1974). The blood Se of new arrivals in New Zealand decreased rapidly at first, to reach the 'New Zealand' range within about 1 year. Some very low blood values (less than 0·030 μg Se/ml) have been found; these are almost within the range associated with Se-responsive diseases in sheep.

The importance of these low values is not clear, but we have been looking at methods for increasing the blood Se of some New Zealanders in the event that it should become necessary. Our dietary intake of 30 μg Se/d or less is about 40–100 μg Se below intakes elsewhere (Griffiths, 1973). This could be increased by eating substantial amounts of liver or kidney or fish daily. We have looked at other methods for supplementing the intake, by dosing with selenite or selenomethionine.

This study reports the dosing of one subject with 100 μg Se as selenomethionine daily for 11 weeks, during which the blood Se increased steadily from 0·08 to 0·18 μg Se/ml. Blood samples were taken twice weekly or weekly, 6–7 h after the dose (Thomson, 1974). The plasma Se increased for the first 6 weeks from 0·07 to 0·15 μg Se/ml, and then remained between 0·14 and 0·16 μg Se/ml. The erythrocyte Se level increased throughout the study, slowly at first, so that the plasma Se was greater than the erythrocyte Se until the last few weeks. Daily urinary Se output followed most closely the plasma Se level, increasing from 12–13 μg Se/24 h to between 35 and 60 μg/24 h, except for days when meals of fish were eaten.

Daily small doses have been found more effective than periodic large doses of 1–2 mg Se as selenite, either in solution, as it is administered to sheep in drenching, or as solid selenite in capsules. Blood samples taken from a group of subjects showed that such dosing had raised the blood Se above the 'New Zealand' range, but most were still well below the blood values of between 0·1 and 0·3 μg Se/ml reported elsewhere (N. M. Griffiths, personal communication).

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Leaf-protein concentrate as a source of minerals. By W. BRAY (introduced by F. AYLWARD), *Department of Food Science, University of Reading, London Road, Reading RG1 5AQ*

In an earlier communication (Bray, 1976) we have emphasized the point that leaf-protein concentrate (LPC) contains a wide variety of substances other than protein and, in particular, significant quantities of vitamins, minerals and lipids. In this paper we report on the content of several inorganic elements in lucerne LPC.

On the basis of our analyses we have calculated the daily contribution from 25 g LPC (the amount supplying 15 g protein to the diet) as a percentage of the (US) recommended daily allowance for 11–14-year-old males ((US) National Research Council, 1974). Based on the material analysed, 25 g LPC would supply more than 100% of the iron requirement, more than 35% of the calcium, magnesium, copper and manganese requirements, more than 25% of the potassium requirement, and a significant amount (more than 10%) of the phosphorus needed.

Of the seven elements considered as macro-nutrients in human nutrition, all except sodium are essential to the growth of green plants. Of the ten micro-nutrients needed by man, half are also required by plants and the others are always found in some concentration in leaves. Thus, it is not unexpected that these minerals would also be found in LPC. The amounts in any one LPC will depend on various factors including the source of raw materials and conditions of cultivation, and also on the methods of extraction and fractionation. The routine quality appraisal of LPC for human use should therefore include analysis for minerals as well as vitamins, pigments and lipids.

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The influence of home diets and eating habits on primary school children's nutritional status. By SUSAN THOMAS and R. C. OSNER, *Department of Hotel and Institutional Management, Sheffield Polytechnic, Pond Street, Sheffield S1 1WB*

The work reported here is part of a wider research programme concerned with the formation of food habits and the nutritional importance of the free school meal.

Questionnaires for completion by parents were distributed to a random sample of 100 children attending Park Hill Primary School in Sheffield, with a catchment area consisting of pre- and postwar council housing; the same number of questionnaires was sent out to Velmead School in an affluent area in Hampshire, for comparison. The children at Park Hill were also interviewed.

Table 1. *Uptake of school meals in two schools*

	Park Hill, Sheffield	Velmead School, Hampshire
Response rate (%)	40	87
Percentage of respondents taking:		
School lunch (paying)	31	86
Free school meal	33	0
Home or packed lunch	17	14
Occasional school lunch	19	0

The findings show that the diet of 'Park Hill' children lacks variety, many families following traditional eating habits. There appeared to be a deficiency of fresh produce such as dairy foods, fruit and vegetables in their diets, and instead many processed foods with synthetic flavours were preferred by the children. This was confirmed by the popularity ratings for school meals.

The questionnaire indicated similar trends in eating patterns, made noticeable against the far wider variety of foods eaten by the 'Velmead' children. For example, the most popular foods mentioned by 'Park Hill' parents included chips, baked beans, sausages and fish fingers; all could be classed as convenience foods, easy to

cook and easy to eat. The most popular foods in Velmead school included dishes such as pizzas, curries, salads and oriental foods.

The heights and body-weights of a sample of children from both Park Hill and Velmead School were compared to recommended statistics for each age group (Watson & Lowrey, 1973). Of the Park Hill children, 45% were found to be below the 10th centile in both their skeletal and body development, whilst only one child from Velmead (1·2%) was below this centile for height and weight-for-age and in addition, 6% were below the 10th centile for one of these two measurements. This suggests that nearly half the children in the Park Hill area of Sheffield may not be reaching their full physical potential and it is probable that the school meal is making a significant contribution to health in this area.

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The effect of dietary tryptophan on the regulation of lipid metabolism in rats. By R. B. FEARS and E. MURRELL, *Beecham Pharmaceuticals, Research Division, Walton Oaks, Dorking Road, Tadworth, Surrey KT20 7NT*

Lipogenesis in the rat is known to be subject to diurnal rhythms which are probably hormonal in origin, although the mechanism of control is unclear. Plasma tryptophan also undergoes a daily variation, with a peak in concentration at midnight (Fermstrom, Larin & Wurtman, 1971), that is, immediately preceding the increase in lipogenesis. Tryptophan, administered intraperitoneally to fasted rats, stimulated hepatic fatty acid synthesis (Sakurai, Miyazawa, Shindo & Hashimoto, 1974).

The present experiments were done to study in more detail the effects of tryptophan on lipid metabolism under physiological conditions. Groups of eight male Sprague-Dawley rats were fed, *ad lib.*, on a stock laboratory diet (Oxoid Ltd, Southwark Bridge Road, London SE1 9HF) with the addition of tryptophan at 5·0, 2·5 or 1·0 g/kg. Control animals received stock diet alone. As nicotinic acid, a known hypolipidaemic agent, is produced by the catabolism of tryptophan (to about 4% of total), another group of rats received nicotinic acid at 0·1 g/kg diet. All groups were killed after 7 d. The control group and the tryptophan-treated (2·5 g/kg) animals received an intraperitoneal injection of tritiated water 1 h before slaughter. The incorporation of ^3H into digitonin-precipitable sterols and total fatty acids in the liver, small intestine and epididymal adipose tissue was measured. Results, given as mean values with their standard errors, were:

Diet	Fatty acid synthesis: mg/liver	Fatty acid synthesis: mg/g small intestine tissue	Fatty acid synthesis: mg/g adipose tissue	Serum triglycerides (mg/l)
Basal	1·29±0·24	0·074±0·007	0·119±0·030	1030±136
+2·5 g tryptophan/kg	2·32±0·27**	0·087±0·008	0·104±0·035	670± 85*

Values significantly different from those for the basal diet: *$P<0·05$, **$P<0·02$.

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Tryptophan increased fatty acid synthesis in the liver but not at other important body sites. Tryptophan had additional metabolic effects to that found on fatty acid synthesis, as shown by the fact that serum triglycerides were reduced in concentration. Minimal changes were found in glycogen and cholesterol metabolism.

The liver is known to have a greater diurnal variation in fatty acid synthesis than is found at other tissue sites and it is the liver that appears to be most sensitive to the effects of tryptophan.

It is suggested therefore that tryptophan participates in the daily regulation of the metabolism of fatty acids. Additional evidence will be presented to support this concept.

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Effects of wheat bran and a mould (*Fusarium*) on cholesterol excretion in rats. By D. E. OWEN, K. A. MUNDAY, T. G. TAYLOR and M. R. TURNER, *Department of Physiology and Biochemistry, The University of Southampton, Southampton SO9 3TU*

The addition of cholesterol (10 g/kg) and sodium cholate (2·5 g/kg) to diets containing cellulose (100 g/kg) as the dietary fibre source and given to male rats for 5 weeks resulted in an increase in the plasma and liver cholesterol concentrations. This increase is prevented or minimized when dietary cellulose is substituted by wheat bran or a *Fusarium* mould (Lord Rank Research Centre, High Wycombe, Bucks.) (Owen, Munday, Taylor & Turner, 1975).

In human studies, no effect of wheat bran on plasma cholesterol concentration has been found in normocholesterolaemic subjects. We have studied, therefore, a similar situation in rats given diets without added cholesterol or bile acids. Male Wistar rats (150 g body-weight) were given *ad lib.* for 5 weeks a diet containing (g/kg) cellulose (100), wheat bran (100) or the mould (400) as the dietary fibre source. Each diet contained an approximately similar amount of dietary fibre. After an overnight fast cholesterol and triglyceride levels were determined in the plasma and liver. There were no differences between the dietary groups in cholesterol concentration of the plasma or liver. The liver triglyceride concentration was higher in the 'bran' and 'mould' groups than in the 'cellulose' group but this only reached significant levels for the 'mould' group. The mean values with their standard errors for six observations were:

Dietary fibre source	Mean food intake† (g/rat per week)	Mean body-wt‡ (g)	Plasma (mg/l)		Liver (mg/g fresh wt)	
			Cholesterol	Triglyceride	Cholesterol	Triglyceride
Cellulose	161·5±3·3	274·0±6·1	717±27	435±46	2·9±0·1	5·7±0·5
Wheat bran	159·9±3·1	*299·2±9·5	737±44	530±46	3·2±0·2	8·9±1·4
Mould	160·7±5·0	*300·1±7·9	775±25	541±37	3·2±0·3	**8·9±0·8

Significance of difference (Student's *t* test) from 'cellulose' groups: $^{*}P<0.05$, $^{**}P<0.01$.
†Mean for the last 4 weeks of the 5-week period.
‡Prior to killing.

The faecal excretion of bile acids (mg/rat per 24 h), was significantly increased in the animals receiving 'bran' (19·1±0·6) and 'mould' diets (10·3±1·0) compared with the 'cellulose' group (6·2±0·3): the faecal sterol excretion was also significantly increased in the 'mould' group. The concentration of both bile acids and faecal sterols (per g dry faecal weight) was significantly higher in 'bran' and 'mould' groups than in the 'cellulose' group. Because the plasma and liver concentrations of cholesterol were not reduced in animals receiving bran- and mould-containing diets it is possible that there was a compensatory increase in the rate of hepatic sterol biosynthesis, as can be implied also from the findings of Morgan, Heald, Atkin, Green & Chain (1974), using bagasse as the source of dietary fibre. Preliminary findings for rats made hypercholesterolaemic by giving a diet containing added cholesterol and bile acids for 4 weeks before testing the effect of different dietary fibre sources suggest that the plasma concentration of cholesterol is reduced more rapidly by wheat bran and the mould than by cellulose, in this situation. It would be interesting, therefore, to know the effect of dietary fibre in hypercholesterolaemic patients.

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The fraction of microbial nitrogen entering the duodenum of sheep.

By J. R. LING and P. J. BUTTERY, *Department of Applied Biochemistry and Nutrition, University of Nottingham, Faculty of Agricultural Sciences, Sutton Bonington, Loughborough, Leics. LE12 5RD*

Nucleic acids have been used previously as markers in attempts to assess the amount of microbial protein entering the duodenum of ruminants. However, the determination of nucleic acids in digesta presents many problems. Numerous interfering substances necessitate extraction procedures that are both time-consuming and tedious.

We have used a method based upon that of Guinn (1966), which is both simple and relatively fast. Freeze-dried samples of mixed rumen bacteria and duodenal digesta were homogenized in ethanol. After a series of extractions in ethanol and ethanol–NaCl, the nucleic acids were extracted from the insoluble fraction by a solution of 100 g NaCl/l at 100°. After precipitation with an ice-cold solution of trichloroacetic acid (100 g/l), the nucleic acids were solubilized in 0·5 M-perchloric acid.

Estimates of the total nucleic acids were made from their extinction at 260 and 280 nm, using a nomogram. The DNA contents of the extracted nucleotides were estimated using the diphenylamine reaction (Burton, 1956) and RNA contents by the orcinol reaction (Kerr & Seraidarian, 1945). The ultraviolet spectra were fairly free from interfering substances and gave values that agreed well with the sum of the obtained DNA and RNA values.

Three sheep, fitted with rumen and re-entrant cannulas, were continuously fed with isoenergetic, isonitrogenous diets, in which about 40% of the nitrogen was derived from either fish meal, urea or soya bean. Estimations of the nucleic acid concentrations in mixed rumen bacteria and duodenal digesta were determined as above. The RNA-nitrogen:total N ratio was, in common with findings of Smith & McAllan (1970), found to be the most suitable measurement for microbial-N estimates. A value of 0·092±0·003 (mean and SE) was obtained for this ratio for rumen bacteria isolated from all sheep on all diets. The results are given in Table 1 and compared with values obtained using a ^{35}S procedure based on that of Harrison, Beever & Thompson (1972) but corrected for the different methionine:N ratios of microbial and dietary proteins.

The RNA method, while taking no account of protozoal protein, gave values generally higher than those obtained by the ^{35}S procedure. Nevertheless both methods produced values of the expected order, that is urea>soya bean>fish meal, with respect to microbial protein production.

Table 1. *Ratio, microbial nitrogen: total N entering the duodenum, determined by measurement of RNA and of ^{35}S, in sheep given isoenergetic, isonitrogenous diets with three different N sources*

(Mean values with their standard errors for three (RNA) or four (^{35}S) observations)

N source ...	Fish meal		Urea		Soya bean	
Sheep no.	RNA	^{35}S	RNA	^{35}S	RNA	^{35}S
1	0·55±0·03	0·52±0·02	0·95±0·10	0·92±0·04	0·56±0·02	0·55±0·02
2	0·58±0·05	0·51±0·03	1·01±0·04	0·94±0·02	0·66±0·06	0·71±0·02
3	0·61±0·04	0·59±0·02	1·06±0·04	0·91±0·03	0·68±0·04	0·66±0·02

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Rates of rumen fermentation in relation to ammonia concentration. By A. Z. MEHREZ and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The minimum ammonia concentration necessary for the maximum rate of rumen fermentation is normally very difficult to assess owing to diurnal variations. To overcome this problem, four rumen-cannulated sheep were fed from an automatic continuous feeder, and were offered daily 1 kg whole barley grains supplemented with minerals and vitamins. Solutions providing 0, 2, 4, 6, 8 and 10 g urea/kg were absorbed into the grains by the method of Ørskov, Smart & Mehrez

(1974). Every sheep received each diet for 1 week except when urea was not included, in which instance the sheep were offered the diet for 3 weeks. On each of the last 2 d of each period, four Dacron bags containing 5 g rolled barley (4·3 g dry matter (DM)), were incubated in the rumen of each sheep at the same time and a bag removed after 1·5, 3, 6 or 9 h of incubation.

NH_3 concentration was measured in the rumen liquor of each sheep initially, and at the end of each incubation interval (five times/d). The absorption method of urea inclusion in the diet and continuous feeding resulted in a relatively steady concentration of NH_3 in the rumen. The mean difference between the highest and lowest values for NH_3 concentrations for the five times of sampling was 24, 43, 29, 53, 52 and 75 mg/l rumen liquor for the diets supplemented with 0, 2, 4, 6, 8 and 10 g urea/kg respectively. Over-all mean NH_3 concentrations for the six diets were 91, 165, 211, 267, 288 and 373 mg/l rumen liquor respectively.

The proportion of DM disappearance from the Dacron bags was described by an equation fitted to the values for each incubation interval from each sheep. The equations were of the form $y=A+Be^{-cx}$, where y is DM disappearance during the incubation interval and x is rumen NH_3 concentration (mg/l rumen liquor). As the NH_3 concentration of the rumen was raised, the total DM disappearance at 1·5, 3, 6 and 9 h increased to a maximum of 0·15, 0·33, 0·48 and 0·60 g/g incubated respectively. The NH_3 concentrations necessary to achieve 0·95, 0·85 and 0·75 of the maximum rate of DM disappearance were (mean and SE): 238±5·3, 186±12·0 and 146±15·5 mg/l rumen liquor respectively.

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A comparison of the efficiency of urea and ammonium salts as non-protein-nitrogen supplements for sheep rations. By N. W. OFFER, R. F. E. AXFORD and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd LL57 2UW*

Four Welsh Mountain×Blackface wethers, equipped with duodenal re-entrant cannulas, were restrained in metabolism cages. They were fed at 2 h intervals, with free access to water. The daily ration was 350 g chopped hay, 200 g barley, 60 g dried grass, 10 g molasses and 10 g minerals and vitamins, fed alone, or supplemented with ammonium lactate, ammonium acetate, or urea. The supplements provided 7 g non-protein-nitrogen (NPN) daily, which doubled the N supplied by the basal diet. The rations provided 129 g modified acid-detergent fibre (MADF) daily. Each sheep received each diet for a period of 14 d. From the 10th to the 14th day, representative samples of digesta were collected from the duodenal cannulas by an automatic device (Axford, Evans & Offer, 1971). Food, digesta, urine, faeces and blood were analysed for energy and nitrogenous constituents.

The results are summarized in Table 1.

The NPN supplements significantly increased the digestibility of MADF, the flow of ammonia-N, non-NH_3-N (NAN) and total amino acids to the duodenum, and led to an increase of approximately 50% in the amount of NAN apparently absorbed from the intestine. The faecal total N was highest with the basal ration.

Ammonium lactate supplementation resulted in the largest increase in amino acid flow from the stomach and the highest NAN absorption from the intestine, but the NPN supplements did not differ significantly ($P>0.05$).

The amino acid profiles of the digesta were examined by the method of constrained optimization (Evans, Axford & Offer, 1975), which showed the NPN supplementation increased the flow of microbial amino acids from the stomach, and that ammonium lactate was superior to ammonium acetate or urea in this respect.

This finding supports the indications from the other measurements that ammonium lactate was the best of the NPN supplements, and that the ammonium salts tested compared favourably with urea.

Table 1. *Effects of non-protein nitrogen supplementation of basal rations for sheep*

	Supplement*				
	None	Ammonium lactate	Ammonium acetate	Urea	SE
Digestibility of MADF	0·38	0·52	0·47	0·52	0·03
Total N intake (g/d)	7·7	14·9	15·5	14·9	—
Passing to duodenum:					
Ammonia N (g/d)	0·3	0·7	0·9	0·8	0·06
NAN (g/d)	10·0	12·6	12·0	12·1	0·55
Total amino acids (g/d)	46·7	60·3	54·9	56·0	2·9
Calculated microbial amino acids (g/d)	17·7	29·8	25·8	26·2	1·1
Total N in faeces (g/d)	4·6	4·0	4·0	4·0	0·2
NAN apparently absorbed from intestines (g/d)	5·4	8·6	8·0	8·1	0·5

MADF, modified acid-detergent fibre; NAN, non-NH_3-N.
*To provide 7 g non-protein-N/d.

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Effect of formaldehyde treatment of dietary casein on amounts and composition of nitrogenous compounds entering the small intestine of the ruminant calf. By A. P. WILLIAMS and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Formaldehyde treatment of dietary protein leads to an increased amount of protein reaching the intestine of ruminants (Faichney, 1974) but the source of this

increased flow has not been established. Calves were given a basal diet of straw and flaked maize (12 g nitrogen/kg dry matter (DM)) or isoenergetic diets with some flaked maize replaced by untreated or formaldehyde-treated casein (10 g formaldehyde/kg protein) to give 19, 26 or 34 g N/kg DM. For the basal diet about the same amounts of total N and amino acid-N (AA-N) entered the duodenum as were eaten, but with increasing supplementation increasing amounts of N disappeared up to the duodenum. This disappearance was considerably less and more AA-N entered the duodenum with treated than with untreated casein; for the highest N intake, for example, the difference in AA-N flow was about 20 g/d.

The amounts of dietary untreated casein N that escaped degradation in the rumen, estimated essentially by the method of McDonald & Hall (1957), were 4·0, 5·7 and 7·4 g/d (0·21, 0·14 and 0·10 as proportions of intake) for diets containing 19, 26 and 34 g N/kg DM respectively. Corresponding values for treated casein were 17·1, 28·7 and 53·8 g/d (0·90, 0·69 and 0·73 as proportions of intake). Amounts of non-casein, non-ammonia-N (mainly microbial and endogenous N) entering the duodenum were 26·7, 37·0 and 42·1 g/d for increasing levels of untreated casein and 20·3, 19·4 and 16·4 g/d for corresponding levels of treated casein. These changes were associated with findings that, compared to the basal diet, untreated casein had little effect on the amino acid composition at the duodenum or on concentrations of plasma amino acids but that treated casein had such effects. At the highest N intake, for example, treated casein altered the proportion of some non-essential amino acids at the duodenum (glutamic acid and proline increased, glycine and alanine decreased) and led to increased concentrations of most plasma essential amino acids. It appears that net changes in AA-N at the duodenum were due partly to survival of dietary protein up to the duodenum and partly to changes in either microbial protein synthesis or endogenous protein secretion.

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Microbiological assay of tryptophan in proteins. By K. J. SCOTT, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Hydrolysis of the test protein, which is a necessary first step in most assay procedures, causes losses of L-tryptophan that are due mainly to racemization but in part to chemical destruction. Peptide-bound tryptophan is readily racemized during alkaline hydrolysis, whereas the free amino acid is comparatively resistant. D-tryptophan is measured in the chemical methods but it has no biological activity for the micro-organisms that are commonly used in microbiological assays.

We found that a satisfactory correction for the losses of L-tryptophan could be made in our microbiological tests by using DL-tryptophan as an external standard in the assay and also as an internal standard to determine the recovery of

tryptophan in the test protein. The test organism was *Streptococcus zymogenes*.

Table 1 shows that results for most of the test proteins agreed closely with those obtained by the chemical procedure (c) of Miller (1967). The values for the purified proteins were close to the theoretical values. Results for the cereals were consistently higher and those for the meat meal were consistently lower. The reasons for these discrepancies are under investigation.

Table 1. *Tryptophan content (g/kg protein or crude protein (nitrogen×6·25)) of various pure proteins and protein feeding-stuffs*

(Mean values; ranges in parentheses)

Samples hydrolysed with . . .	Microbiological test		Chemical test
	Barium hydroxide	Sodium hydroxide	Barium hydroxide
Lysozyme (*EC* 3.2.1.17)	91·6 (83·2–99·9)	—	89·7 (94·2, 85·2)
α-Chymotrypsin (*EC* 3.4.4.5)	58·9 (51·3–64·3)	—	62·2 (59·1, 65·3)
Haemoglobin	19·5 (19·7, 19·3)	—	21·0
Fish meal 104*	8·6 (8·3, 8·9)	8·7 (8·1–9·4)	8·9 (8·3–9·5)
Soya-bean meal	14·8 (13·7, 16·0)	14·8 (14·3–15·2)	14·3 (13·4–14·8)
Meat meal 101*	7·5 (8·0, 7·0)	7·5 (7·2–7·7)	9·2 (8·4–10·4)
Groundnut meal 101*	10·2	11·2 (10·5–11·9)	10·8 (10·0–11·9)
Wheat	13·8 (13·4–14·2)	—	11·5 (11·7, 11·4)
Barley	14·2 (13·5–14·6)	—	10·5 (10·3, 10·7)

*From ARC Protein Evaluation Group series (see Boyne, Ford, Hewitt & Shrimpton, 1975)

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Determination of lysine in barley by a modified dye-binding procedure.

By G. P. JONES* and A. L. LAKIN (introduced by F. AYLWARD), *Department of Food Science, University of Reading, Reading RG1 5AQ*

The association of acid azo dyes with proteins at low pH is mainly dependent upon the association between dye molecules and the functional groups of basic amino acids in the protein molecules. Accordingly, high correlations have been demonstrated between the dye-binding properties of cereals and their total content of basic amino acids and also, but to a lesser extent, their contents of lysine (Mossberg, 1969).

In order to improve the accuracy of dye-binding procedures for the estimation of lysine and possibly to permit their use for the direct determination of this amino acid, modifications which employ lysine-specific reagents are being studied (Jones, 1974). The basis of this work is to measure the dye-binding properties of food materials before and after 'blocking' the ε-amino group of lysine with these

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reagents. The reduction in the amount of dye bound on account of this treatment, designated the 'dye-binding difference' (DBD), is being studied as a measure of the content of 'reactive' lysine.

The experimental procedure applied to barley was as follows. A sample (about 1 g, ground to pass a 0·5 mm mesh) was incubated with 20 ml of a solution containing 10 g 2, 4, 6-trinitrobenzene sulphonic acid/l 0·238 M-sodium hydrogen carbonate for 5 h at 40°. The treated material was separated by filtration, agitated overnight with 50 ml dye–buffer reagent (1·75 mmol Acid Orange 10/l 0·2 M-citric acid) and the amount of dye absorbed was calculated from spectrophometric measurements. The amount of dye bound by an untreated sample was similarly determined and the DBD value of the material found by difference.

When this procedure was applied to a series of twenty-four feed-barley samples (1971 harvest) there was a strong correlation (r 0·951) between their DBD values and their content of lysine, as determined by amino acid analysis. The mean DBD value (calculated as lysine) for the series was 4·75 mg/g and the mean lysine content was 4·40 mg/g (SE of estimate 0·33).

Studies are now being made of the application of this principle to other food materials; and in particular with respect to the use of alternative lysine-specific reagents and dyes other than Acid Orange 10.

Since we started this work, a similar procedure has been reported (Sandler & Warren, 1974) in which Acid Orange 12 is used as the dye and ethyl chloroformate as the lysine-specific reagent.

We wish to thank the Agricultural Research Council for supporting this work and the Plant Breeding Institute, Cambridge, for providing analysed samples of barley.

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Myofibrillar protein breakdown in the rat. By L. C. WARD, P. J. BUTTERY and K. N. BOORMAN, *Department of Applied Biochemistry and Nutrition, University of Nottingham, Faculty of Agricultural Sciences, Sutton Bonington, Loughborough, Leics. LE12 5RD*

The amino acid 3-methyl histidine (3-MeHis) is a characteristic constituent of the skeletal and cardiac myofibrillar proteins actin and myosin (Asatoor & Armstrong, 1967; Johnson, Harris & Perry, 1967) and is not incorporated into proteins directly, but is formed by the methylation of histidine. Once released, on the breakdown of myofibrillar protein, 3-MeHis is not re-utilized, but is excreted in the urine (Young, Alexis, Baliga, Munro & Muecke, 1972). Therefore estimation of the rate of 3-MeHis release by muscle should be an index of the true rate of

myofibrillar protein breakdown. Furthermore, if the 3-MeHis residues can be isotopically labelled, comparison of the specific activities of 3-MeHis in muscle protein and in urine should provide information on the kinetics of myofibrillar protein breakdown.

Male, specific pathogen-free Wistar rats (180–200 g body-weight) were injected intraperitoneally with 400 μCi [*Me*-^{3}H]methionine, placed in stainless-steel metabolism cages and fed *ad lib.* The urine was collected. At various time intervals after injection the hind-limbs of the rats were perfused using a method based on that of Ruderman, Houghton & Hems (1971). The specific activity of 3-MeHis was determined in the urine, trichloroacetic acid-precipitable muscle protein, and in the perfusate using preparative ion-exchange chromatography.

Fractional decay rates and half-lives of mixed myofibrillar proteins were calculated from the total daily 3-MeHis output and the total body skeletal muscle or perfused muscle 3-MeHis pool. First-order kinetics were assumed.

Daily urinary 3-MeHis output was relatively constant (2·30±0·03 μmol/d (mean±SE; *n* 6)) and indicated a fractional rate of myofibrillar protein degradation of 0·037±0·002/d, corresponding to a half-life of 18·57 d. The increase in the perfusate concentration of 3-MeHis was similar, indicating a fractional decay rate of 0·076±0·006/d (half-life 9·54 d). The differences in the specific activity of 3-MeHis in muscles of the hind-limb, urine and perfusate (Table 1) may confirm the suggestion that myofibrillar protein breakdown may not follow single exponential kinetics (Haverberg, Omstedt, Ljungqvist, Steinert, Munro & Young, 1974).

Table 1. *Specific activity (disintegrations/min per μmol (×10^{-4})) of 3-methyl histidine in muscle, urine and hind-limb muscle perfusate of rats*

	Time after injection (d)					
	1	2	4	6	8	10
Muscle	2·0	1·7	2·2	1·7	1·4	1·5
Urine	6·3	5·9	2·4	2·5	1·4	1·5
Perfusate	7·9	7·0	6·3	5·8	6·2	5·6

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Muscle and liver protein metabolism in rats treated with glucocorticoids.

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The mechanism by which glucocorticoids mobilize muscle protein for gluconeogenesis is not understood. We have studied therefore the in vivo effect of triamcinolone acetonide on skeletal muscle and liver protein synthesis and on the protein and RNA content of these tissues. When administered in daily intraperitoneal injections (5 mg/kg body-weight), the steroid stopped the growth of 100 g male rats given a good diet, increased the liver protein and RNA content, but had variable effects on muscle (quadriceps and gastrocnemius). In some experiments there was a loss of protein (20 % in 4 d) while in others no muscle protein was lost. A common finding, however, was a loss of total muscle RNA (e.g. 33 % in 4 d). The rate of muscle protein synthesis measured by the constant infusion of [^{14}C]tyrosine (Garlick, Millward & James, 1973), decreased from 12·9 %/d in the growing untreated rats to 10·8 %/d ($P<0·05$) after 4 d of the steroid treatment. This decrease largely reflected the cessation of growth and there was no indication that the catabolic rate was changed. Measurement of plasma insulin concentrations at the end of the infusion (i.e. after a 6 h fast) revealed that in the steroid-treated rats, insulin concentration was invariably very high, i.e. 50–100 μunits/ml compared with 15 μunits/ml in untreated rats.

Because insulin and glucocorticoids might be expected to have opposing effects on muscle protein metabolism, an attempt was made to reduce the insulin secretion in response to the glucocorticoids by giving the steroids to malnourished rats. Rats were given a diet containing 35 g protein/kg for 1 month and then given daily injections of the steroid for 4 d, while being given the low-protein diet. At the start of the steroid treatment the malnourished rats had reduced muscle RNA concentrations and reduced rates of protein synthesis (5·8 %/d). During 4 d of the steroid treatment there was no change in the muscle protein and RNA content or in the rate of protein synthesis. However, liver protein and RNA content increased in each instance in response to the steroid treatment and the rate of protein synthesis increased from 69 %/d to 95 and 111 %/d after 2 and 4 d respectively. The low-protein regimen did not suppress the insulin release in response to the steroids. The plasma insulin concentration increased from 7·7 μunits/ml in the untreated, malnourished rats to 35 μunits/ml in the treated animals. Plasma glucose concentrations were also increased.

These results suggest that because of the increased insulin secretion following glucocorticoid administration, the role of these steroids in muscle protein mobilization cannot be evaluated in an experimental design as described here.

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Estimation of muscle leucine oxidation in the perfused hind-limb of the rat: effect of feeding with a protein-free diet. By R. D. SKETCHER and W. P. T. JAMES*, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Recent studies have shown that total branched-chain α-oxo-acid dehydrogenase activities in skeletal muscle exceed those in liver (Reeds, 1974; Sketcher, Fern & James, 1974) and that this activity can change in response to dietary intake of protein (Sketcher & James, 1974). Muscle could therefore be an important site not only for deaminating the branched-chain amino acids but also for oxidizing the carbon chains. No attempt has been made yet to quantitate the contribution of skeletal muscle to total body leucine oxidation. The present work compares the oxidation rate of leucine measured in the perfused hind-limb from rats given normal or protein-free diets for 1 week. Oxidation rates are determined from the output of $^{14}CO_2$ from L-[1-^{14}C]leucine given in a tracer dose by continuous infusion for 2 h (Waterlow & Stephen, 1968; Fern & Garlick, 1973).

Protein-free feeding led to a reduced rate of oxidation from 4·85 to 2·03 μmol/h in the perfused hind-limb (Table 1). This lends support to the proposed role of skeletal muscle in the control of leucine oxidation made from in vitro enzyme measurements (Sketcher & James, 1974).

Table 1. *Oxidation rates of* L-[1-^{14}C]*leucine in the perfused hind-limbs from rats given a normal diet (*NDp:E 0·10*) or protein-free diet (*NDp:E 0*) for 1 week*

(Mean values and standard deviations)

NDp:E of diet	No. of rats	Wt (g)		$^{14}CO_2$ output at 2 h (disintegrations/min per min infusion)	SR of free leucine in gastrocnemius muscle at 2 h (disintegrations/min per μmol leucine)	Oxidation rate of leucine at 2 h (μmol/h)
		Rat	Hind-limb			
0·10	5	182·7± 8·2	67·7±6·0	7008±256	90 814±23 951	4·85±1·12
0	4	178·4±17·1	69·2±7·6	4018±225	113 837±18 697	2·03±0·61**

NDp:E, ratio, energy supplied by utilizable protein:total metabolizable energy; SR, specific radioactivity.

Value differs significantly from that for normal diet: **$P<0·005$.

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The stationary nature of variation in daily nitrogen balance. By P. V. SUKHATME, *Maharashtra Association for Cultivation of Science, Poona-4, India*

The results of recent studies by Scrimshaw and his associates (Scrimshaw, Hussein, Murray, Rand & Young, 1972) purport to indicate that, contrary to the view expressed by Holmes (1965), a stable rate of obligatory nitrogen output is reached in adults in 3–7 d if an adequate energy intake is provided to the subject. Re-examination of their results suggests that their conclusion is valid only if the obligatory N output on successive days is uncorrelated. There is no means of studying the validity of this assumption in their results, but similar results for N losses with a low protein intake reported by Calloway & Margen (1971) suggest that N balance on successive days is serially correlated; the correlation coefficient found was greater than 0·5 (see Table 1). Further the serial correlation was found to decrease as the lag period increased.

The same result is found when the subjects are given a higher N intake; the serial correlation being even higher than that found with the protein-free diet. The conclusion is, therefore, that the fluctuations in the daily output of N with a protein-free diet are neither purely random nor do they represent a smooth oscillatory movement, but that they represent a situation between the two, with the series showing irregular amplitudes and irregular distances between peaks and troughs and yet along with this irregularity of movement, there is some kind of systematic effect. As Table 1 indicates, this systematic effect can be adequately described by the Marcoff system. The deviations from this system are integrated into the motion of the system, giving it a momentum to ensure that the future behaviour of N output will be like that in the past, in the probability sense, i.e. unlike the conclusion of Scrimshaw *et al.* (1972), the output will not stabilize to a fixed value. Instead, the daily obligatory N losses will behave like a stationary time series, varying about a constant mean value with a constant variance and covariance with time. The result is true regardless of whether the variation relates to 'day-to-day' losses or to fixed periods such as 3, 5 or 7 d. As the period (n) over

Table 1. *Variance of oscillation in nitrogen balance*

N intake (g)	Lag period (d)	Serial correlation coefficient		Series	Variance (g^2)	
		Subject no. 4	Subject no. 6		Subject no. 4	Subject no. 6
0·64	0	1·000	1·000	Original	0·158	0·079
	1	0·551	0·614	Markoff	0·110	0·049
	2	0·397	0·435	Yule	0·108	0·049
	3	0·104	0·212	—	0·102	0·049
		Subject no. 3	Subject no. 5		Subject no. 3	Subject no. 5
3·50	0	1·000	1·000	Original	0·588	0·444
	1	0·706	0·669	Markoff	0·295	0·245
	2	0·576	0·467	Yule	0·288	0·245
	3	0·477	0·411	—	0·287	0·220

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which the losses are averaged increases, the variance will undoubtedly decrease, not inversely as n, as is generally assumed, but approximately as $\frac{1}{n}\left(\frac{1+\rho}{1-\rho}\right)$, where ρ is the serial correlation coefficient. Consequently, even with a correlation coefficient as small as 0·5 the 'within-variance' based on 5 d average values will contribute appreciably to the total variation. The implications of these findings for formulating models for protein deficiency will be discussed.

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