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ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Thirty-fourth Scientific Meeting of the Nutrition Society was held in the Royal Society of Medicine, 1 Wimpole Street, London W1, on Friday, 21 May 1971, at 10.45 hours, when the following papers were read :

Rate of adaptation of some of the urea cycle enzymes to a low-protein diet. By T. K. DAS, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine London, WC1*

It has been demonstrated by Waterlow & Stephen (1966) that when rats are given diets containing less than the normal content of protein they become adapted to the reduced protein supply by an alteration in the pattern of protein synthesis. Schimke (1962) has shown that, when the diet contains no protein but enough calories to provide energy, the levels of urea cycle enzymes fall, corresponding to the decrease in urea excretion. Mariani, Migliaccio, Spadoni & Ticca (1966) found that under these conditions the activity of the amino acid synthetases in the liver increases. These two groups of enzymes seem to promote the adaptation to a low-protein intake by changing their concentrations in opposite directions. Thus, there appears to be an adaptation of enzyme activity to meet the demands of the body in response to changes in diet (Waterlow, Alleyne, Chan, Garrow, Hay, James, Picou & Stephen, 1966).

The present study is concerned with the rate of adaptation of arginase, argininosuccinase and argininosuccinate synthetase to low-protein feeding, since it was not clear from the work quoted whether the enzyme changes occur rapidly enough to be regarded as causing the change in urea output.

Male black and white hooded rats of similar body-weight were divided at 5 weeks of age into three groups. Groups A and B were maintained on a diet containing 14% protein and group C on a diet containing 5% protein for 2 weeks. Both diets were similar in every respect except that in C the protein was replaced by maize starch. After 2 weeks group B animals were transferred to the 5% protein diet. From this time on, 3 g food were given to each rat at 6 h intervals and urine was collected by washing the metabolic cages into 10 ml M-HCl. Rats of each group were killed at 6 h intervals and the livers were immediately frozen in solid CO_2 and then stored in the deep-freeze.

The measurements of urinary nitrogen suggest that on reducing the dietary protein adaptation is complete in 30 h. The rate of adaptation of the three enzymes studied was similar and the final levels were reached after 30 h.

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The effect of dietary protein and carbohydrate on the intestinal transport of histidine and galactose. By R. M. GOLDSMITH, K. A. MUNDAY and M. R. TURNER, *Department of Physiology and Biochemistry, University of Southampton*

The concept that intestinal absorption of a specific nutrient is affected by its level in the diet has received very little attention since it was suggested by Donnhofer (1943). The work reported in this paper suggests that the intestinal amino acid and sugar transport systems respond to changes in dietary protein and carbohydrate levels.

Groups of animals were fed for 6 d on one of several diets in which the levels of casein and carbohydrate were varied between 0% and 75%. The absorption of L-[^{3}H]histidine and D-[^{3}H]galactose was measured in vivo from short loops of intestine. The results are shown in the table.

Table 1. *The transport of histidine and galactose in vivo from the small intestine of rats receiving varying levels of dietary protein and carbohydrate*

(Mean values with their standard errors for twelve rats)

Composition of diets (% dry wt):				
Casein	0	25	50	75
Carbohydrate	75	50	25	0
Intestinal transport:				
Histidine (μmol/g wet gut per h)	172±6	194±11	208±6**	231±18**
Galactose (μmol/g wet gut per h)	262±10	244±11	214±7**	195±12***

Significance of difference (t) from 0% casein diet: *$P<0.01$; ***$P<0.001$.

The increase in dietary protein from 0% to 75% of the diet with a simultaneous decrease in carbohydrate from 75% to 0% was associated with a 29% increase in histidine transport per g tissue and a simultaneous 19% decrease in galactose transport. The high level of galactose transport in the rats receiving low levels of dietary protein suggests that the effect on histidine transport was a specific one and not just the consequence of a protein insufficiency.

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Effect of degraded and undegraded alginates on the colon of guinea-pigs.

By J. WATT, *Department of Pathology, University of Liverpool* and R. MARCUS, *Clatterbridge Hospital, Bebington* (introduced by G. A. J. PITT)

Ulceration of the colon in guinea-pigs is readily produced by including in their drinking-water carrageenin derived from the red seaweed *Eucheuma spinosum*. Degraded carrageenin is even more ulcerogenic than the undegraded product. Like carrageenin, alginates—also of seaweed origin—are widely used as a food stabilizer and possess some of the physico-chemical properties of the carrageenins. In view of this, an investigation was carried out to determine the effects, if any, of alginates on the colon of the guinea-pig.

Adult male albino guinea-pigs were fed on a standard cube diet (Oxo SG1) supplemented with fresh cabbage and hay. Five guinea-pigs were given a 1% aqueous solution of sodium alginate (BDH Chemicals Ltd) as drinking fluid over a period of 10 weeks; five control animals received water without alginate. Two groups of six guinea-pigs were given 1% aqueous solutions of food-quality sodium alginate (Alginate Industries) for a period of 7 months; one group received Manucol SA/DN, a high-viscosity sodium alginate extracted from the brown seaweed *Ascophyllum nodosum*; the other group received Manucol SS/LD, a low-viscosity preparation obtained from *Laminaria hyperborea*, that was degraded by heating the moist alginic acid before neutralization to give sodium alginate; six control animals received water without added Manucol.

Allowing for spillage from drinking bottles, the average daily consumption per animal of sodium alginate (BDH), Manucol SA/DN and Manucol SS/LD was less than 0·18 g, 0·13 g, and 0·22 g/100 g body-weight, respectively.

At the end of 10 weeks the average weight gain ($\pm$SE) of the guinea-pigs receiving sodium alginate (BDH) was 335 ± 46 g (control animals, 362 ± 48 g). At the end of 7 months the average weight gains of the guinea-pigs receiving Manucol SA/DN and Manucol SS/LD were 300 ± 47 g and 558 ± 92 g respectively (control animals 310 ± 41 g). All the animals appeared healthy, having gained weight satisfactorily. They showed no diarrhoea or occult blood in the faeces.

At the end of the experimental period, the animals were killed by ether anaesthesia; the large bowel was emptied of faeces and carefully examined using transmitted light. No ulceration was found in any of the animals given degraded or undegraded sodium alginate.

The buccal absorption of vitamin C. By A. ODUMOSU and C. W. M. WILSON, *Department of Pharmacology, University of Dublin, Trinity College, Dublin, Ireland*

Ascorbic acid (AA) solution (1 mg/25 ml distilled water) buffered at various pH values was introduced into the mouth as described by Beckett & Triggs (1967) for varying periods. AA was measured in the expelled mixture and in the buccal mucosal cells (BMC) scraped from the inner surface of the cheek by the phenylhydrazine

method of Denson & Bowers (1961) for leucocytes. Salivary pH was similar in both sexes (6·17 ± 0·37). Male and female centrifuged saliva contained 1·77 ± 0·23 and 1·76 ± 0·51 μg AA/ml respectively. Uncentrifuged saliva from the same subjects contained 8·34 ± 0·63 and 12·83 ± 1·46 μg/ml respectively. Uncentrifuged saliva contained leucocytes and mucosal cells. Under normal conditions, male and female BMC contain 22·31 ± 1·98 and 28·57 ± 4·90 μg AA/g respectively ($P < 0·05$).

AA disappearance was a function of retention time and pH of the solution (Table 1) and decreased in both sexes after 5 min, when about 75% had been absorbed. After any interval and at all pH values, AA disappearance was greater in males than in females, and a larger quantity had been absorbed into the male BMC.

Table 1. *Effect of contact time and pH of ascorbic acid (AA) solution on disappearance of AA from the mouth and its uptake by buccal mucosal cells (BMC) in human subjects*

(Mean values and standard deviations)

		Males		Females	
pH	Contact time (min)	From mouth (μg)	BMC uptake (μg/g)	From mouth (μg)	BMC uptake (μg/g)
3·4	1	110·33 ± 11·46	2·71 ± 0·17	74·80 ± 9·32	1·43 ± 0·14
	2	132·38 ± 13·04	3·73 ± 0·18	112·70 ± 11·71	2·92 ± 0·31
	3	368·25 ± 29·84	4·77 ± 0·17	309·57 ± 24·42	3·65 ± 0·41
	4	572·42 ± 29·40	0·24 ± 0·31	507·27 ± 15·01	4·95 ± 0·60
	5	816·62 ± 12·15	9·74 ± 0·26	706·58 ± 18·38	8·06 ± 0·36
	9	883·57 ± 14·97	10·56 ± 0·40	799·80 ± 17·58	9·09 ± 0·47
3·4	5	826·6 ± 43·2	11·5 ± 0·9	727·6 ± 32·8	8·8 ± 0·5
6·0	5	492·2 ± 24·4	7·0 ± 0·4	443·6 ± 18·7	6·4 ± 0·5

Raising the pH reduced disappearance of AA from the mouth and diminished BMC uptake. At normal salivary pH only 50% of AA was absorbed after 5 min. The rates of disappearance of AA L- and D-isomers from the mouth were not significantly different. Because of this, and because contact time of the ionized lipid-soluble form with the buccal membrane determined its percentage disappearance, disappearance of AA can be ascribed to diffusion into the lining lipid mucous membrane. Examination of the BMC scrapings demonstrated the partition of AA into the cells as it disappeared from the cavity. Normally, higher AA concentrations occur in female cells and plasma (Loh & Wilson, 1971). The cell content of uncentrifuged saliva explains its high AA concentration and also the higher female salivary levels. AA entered the BMC, and passed into the plasma, in greater concentrations in males than in females because the diffusion gradient across the male cell was higher, on account of the temporarily raised concentrations in the mouth and the initially lower male cell levels.

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The effect of nematode parasitism on the retention of calcium and phosphorus by growing lambs. By A. E. REVERON, J. H. TOPPS, G. PRATT and A. L. GELMAN, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

At a recent meeting of the Society, results were presented by Reveron, Topps & Pratt (1971) which showed that infestation with *Trichostrongylus colubriformis* of growing lambs adversely affected their bone mineralization. In order to ascertain whether the lambs were suffering from a deficiency of either calcium or phosphorus, balance studies were carried out at three stages of growth. The results of the third balance study, made 2–3 weeks before the animals were slaughtered, are shown in Table 1.

Table 1. *Calcium and phosphorus balance (g/24 h) of control lambs and lambs infested with* 40 000 *larvae of* Trichostrongylus colubriformis

(Mean values for six, nine and eight animals respectively)

	Calcium			Phosphorus		
Group	Intake (I)	Faeces (F)	(I-F)	Intake (I)	Faeces (F)	(I-F)
Control	12·88	5·52	7·36	5·60	4·30	1·30
Infested, growth slightly retarded	10·53	5·21	5·32	4·50	4·10	0·40
Infested, growth severely retarded	4·49	3·25	1·24	1·50	1·40	0·10

The infestation appeared to cause a marked decrease in the apparent absorption and retention of phosphorus but had little or no effect on calcium metabolism. In a subsequent experiment similar lambs given the same parasitic infestation were randomly divided into groups which received either cholecalciferol or phosphorus or both nutrients, or no supplement. Balance studies were made on the lambs at three stages of growth and the results of the third balance are shown in Table 2.

Table 2. *Calcium and phosphorus balance (g/24 h) of control lambs and lambs infested with* 40 000 *larvae of* Trichostrongylus colubriformis

(Mean values for four animals)

	Calcium				Phosphorus			
Group	Intake	Faeces	Urine	Retention (% of intake)	Intake	Faeces	Urine	Retention (% of intake)
Control	10·66	3·97	0·025	62	4·50	2·40	0·045	46
Infested	11·17	4·83	0·035	56	4·75	3·50	0·238	21
Infested+ cholecalciferol	10·38	4·13	0·025	60	4·25	3·50	0·015	17
Infested+ cholecalciferol +P	10·56	4·34	0·044	58	7·00	5·50	0·207	18
Infested+P	11·16	4·53	0·027	59	7·25	5·00	0·179	29

The lack of bone mineralization in the infested animals was less severe than that found in the previous experiment. In comparison with the other two groups, lambs

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not given extra phosphorus had bones of poorer quality, as shown by radiography, and a lower retention of phosphorus.

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The value of different sources of nitrogen in diets for the early-weaned calf. By M. KAY, A. PAVLIČEVIĆ, N. A. MACLEOD and R. SMART, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Experiments with young calves (Kay, MacLeod, McKiddie & Philip, 1967) demonstrated that urea was used less efficiently as a nitrogen supplement by young calves than was white fish meal. The poorer utilization of urea may be due either to its rate of hydrolysis to ammonia being faster than the rate of incorporation of the ammonia into bacterial protein, or to a deficiency in the amount or quality of the bacterial protein produced.

To examine further the poorer utilization of the urea, four Friesian calves each fitted with permanent cannulas in the abomasum and the terminal ileum were offered a basal diet containing either cereals alone (B), or with additions of 1·9% urea (BU), 8·4% white fish meal (BF), or 1·9% urea together with 8·4% white fish meal (BUF). The basal cereal diet contained 10·2% crude protein in the dry matter and the diets were offered twice daily at the same level of feeding for each calf during each period. A Latin square design was used for three weekly feeding periods and samples of digesta were taken from the abomasum and ileum at 2 h intervals over 24 h during the 18th and the 21st day of each period. To estimate the amount of bacterial protein passing through the abomasum, diaminopimelic acid (DAPA) was determined on rumen bacterial preparations obtained from calves given each diet and on the abomasal samples. Chromic oxide impregnated in paper, given in capsules, was used as an indigestible marker.

The DAPA content, per 100 g microbial N, varied from 3·19 g (diet B) to 4·47 g (diet BF). The main results are given in Table 1.

Table 1. *Nitrogen intakes and N passing through the abomasum and ileum of calves given the basal diet either alone (B) or with urea (BU) or fish meal (BF) or both (BUF)*

Dietary treatment	N intake (g/d)	N passing abomasum (g/d)	Proportion of bacterial N in abomasal N (%)	N passing ileum (g/d)
B	27·5	44·5	100	16·0
BU	45·5	54·5	96	16·0
BF	43·0	54·5	56	17·5
BUF	63·0	55·0	60	18·0

On the basal diet, at least 17 g N were recycled to the stomach daily and all the N recovered from the abomasum was of bacterial origin. The urea N gave more

bacterial growth than was observed in the calves on treatment B. The results suggest that the N of fish meal was less available for utilization by bacteria than was urea N or cereal N. Nevertheless, there was the same amount of N passing through the abomasum and the same net absorption of N from the small intestine when urea and fish meal were offered alone or in combination, thus demonstrating that differences in the nature of the nitrogenous components leaving the rumen are most likely to be responsible for the poorer growth of young calves fed on diets containing urea.

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Chemical and in vitro digestion procedures for the prediction of the digestibility of forage crops by sheep. By D. F. OSBOURN, R. A. TERRY, G. E. OUTEN, S. B. CAMMELL and P. R. LANSLEY, *Grassland Research Institute, Hurley, Maidenhead, Berkshire*

Timothy, perennial ryegrass, Italian ryegrass, sainfoin and whole-crop maize were each harvested on nine successive occasions in primary growth and the herbage was frozen and stored at $-20°$. The forty-five herbages were each fed, at levels calculated to meet maintenance requirements for energy, to three mature wether sheep harnessed for faecal collection. The mean coefficients of apparent digestibility of the dry matter of the forages (DMD), determined in vivo, were predicted with high precision by the two-stage in vitro digestion procedure of Tilley & Terry (1963), with reasonable reliability by the two-part prediction equation based upon chemical analyses of the herbage (Van Soest, 1965), and with low precision by simple determination of the content of lignocellulose, measured as the material insoluble in acid detergent solutions (ADF: Van Soest, 1963).

(1) $\text{DMD} = 0{\cdot}26 \pm 0{\cdot}612 + 1{\cdot}010 \pm 0{\cdot}0299$ DMD (in vitro) r^2 0·95; RSD $\pm 1{\cdot}468$

(2) $\text{DMD} = 11{\cdot}32 \pm 3{\cdot}064 + 0{\cdot}850 \pm 0{\cdot}0428$ DMD (Van Soest) r^2 0·90; RSD $\pm 2{\cdot}163$

(3) $\text{DMD} = 101{\cdot}64 \pm 2{\cdot}452 - 0{\cdot}906 \pm 0{\cdot}0779$ ADF r^2 0·77; RSD $\pm 3{\cdot}230$

A further nine red clover herbages were included in this study and equation (4), corresponding to equation (2), was calculated for the fifty-four herbages:

(4) $\text{DMD} = 9{\cdot}02 \pm 3{\cdot}258 + 0{\cdot}882 \pm 0{\cdot}0455$ DMD (Van Soest) r^2 0·88; RSD $\pm 2{\cdot}368$

There was close agreement between this study and that of Van Soest (1965) regarding the first part of the summative equation which predicts the digested cell contents (DCC) from estimates of the cell contents (CC) in the herbage (equation 5):

(5) $\text{DCC} = 0{\cdot}96 \pm 0{\cdot}018\ \text{CC} - 11{\cdot}1 \pm 0{\cdot}97$ r^2 0·98; RSD $\pm 1{\cdot}475$

The errors attached to equation (4) appear therefore to result from errors in the second part of Van Soest's equation which predicts the digestibility of the cell-wall constituents from the logarithm of the concentration of lignin in the acid detergent fibre, and which in this study accounted for only 89% of the variance in digestibility of the cell-wall constituents (RSD $\pm 4{\cdot}071$). Summative two-part equations

(6) and (7) were calculated by separate estimation of the two parts from the values in this present study. These equations related in vivo estimates of DMD to laboratory estimates of the cell contents (CC), cell-wall constituents (NDF), acid detergent fibre (ADF) and acid insoluble lignin (ADL), and in vivo estimates of D-value (digested organic matter as % of the dry matter) to the corresponding ash-free estimates CCA, NDFA and ADFA in the herbage dry matter.

(6) DMD = 0·96 CC − 11·1 + NDF (1·312 − 0·635 log ADL × 100/ADF)

(7) D-value = 1·01 CCA − 10·5 + NDFA (1·353 − 0·654 log ADL × 100/ADFA)

The residual standard deviation of the regression equations predicting in vivo estimates of digestibility from the values calculated from equations (6) and (7) were ±2·37 and ±2·42. It was therefore concluded that the use of these equations as an alternative to in vitro digestion measurements is more precise than prediction based upon estimates of the content of acid detergent fibre (equation 3).

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The effects of drying and the comminution of red clover on its subsequent digestion by sheep. By D. E. BEEVER, D. J. THOMSON and D. G. HARRISON, *Grassland Research Institute, Hurley, Maidenhead, Berkshire*

Previous studies (Thomson, Beever, Coehlo da Silva & Armstrong, 1969) have shown that grinding and pelleting a dried lucerne caused a marked reduction in the proportion of energy and cellulose digested within the reticulo-rumen, but with no change in total digestion of these components. The effect of drying alone upon the digestion of a grass forage (Beever, Thomson, Pfeffer & Armstrong, 1969) was to depress the apparent digestibility of the crude protein but to increase the quantity of amino acids entering and being digested within the small intestine.

In order to investigate further aspects of drying and alterations of physical form, a tetraploid red clover (*Trifolium repens* var. Hungaropoli) was harvested in primary growth on 13 June and conserved in three forms, namely: (1) frozen, by blast-freezing the fresh-chopped material at −21° for 48 h, (2) wafers, by compressing the chopped material after drying into semicircular discs, and (3) pellets, by grinding the dried chopped material through a 1·96 mm sieve before pelleting. Each of the three forms was fed at 900 g dry matter/d in two feeds to three sheep fitted with re-entrant cannulas at the proximal duodenum and terminal ileum (MacRae & Armstrong, 1969). Rumen volatile fatty acid (VFA) production rates were determined by the method of Weller, Gray, Pilgrim & Jones (1967) using ^{14}C-labelled sodium acetate.

Grinding the forage did not markedly influence the total digestion of energy and cellulose but did reduce the proportion of the digested energy lost within the rumen (Table 1). Similarly, the quantity of digestible cellulose lost in the rumen

was reduced from 96% ±1·07 on the frozen and wafered diets to 77% ±4·08 on the pelleted diets. Further confirmation of this reduced digestion occurring within the rumen was obtained from the VFA production rate measurements which were 4·45, 4·54, and 3·65 (±0·05) mol/d for the frozen, wafered and pelleted diets respectively.

The apparent digestibility of total nitrogen was reduced in sheep on both dried forms of the diet (see Table 1), but quantities of total amino acids entering the

Table 1. *The disappearance of energy, as % of the apparently digestible energy (ADE), in different sections of the gastro-intestinal tract, and the mean quantities (g/24 h) of total amino acids present in the feed and faeces and passing at the duodenum and ileum of sheep fed the three red clover diets*

	Frozen	Wafers	Pellets	SEM
Apparent digestibility of gross energy (%)	68·4	64·6	66·0	0·53
Disappearance of ADE (%)				
(a) before small intestine	63·0	60·2	47·5	2·71
(b) in small intestine	32·4	29·1	38·7	4·54
(c) in caecum and colon	4·6	10·7	13·8	1·93
Apparent digestibility of N (%)	72·0	62·1	66·4	1·00
Total amino acids (g/24 h) present:				
In feed	127·2	123·7	122·9	
At duodenum	132·7	147·6	175·2	2·74
At ileum	38·5(71)[1]	69·4(53)[1]	79·5(55)[1]	2·01
In faeces	28·5(26)[2]	50·8(28)[2]	42·4(46)[2]	1·36

Figures in parentheses refer to the disappearance of total amino acids within the small intestine (1) and the large intestine (2) as % of the amount entering the specified section of the tract.

small intestine were greater than with the frozen diet, the most marked effect being due to grinding and pelleting. However, the digestibility of the amino acids within the small intestine with the two dried diets (54%) was much lower than with the frozen diet (71%), with the net result that the losses of total amino acids within the small intestine were: frozen, 94·2; wafers, 78·2; and pellets, 95·7 g/d (±4·4). The losses for methionine were 1·6, 3·0 and 2·6 g/d ±0·03 respectively, and for leucine 8·5, 8·5, and 13·4 g/d ±0·44 respectively, indicating some effect of drying and physical comminution of the diet and also that the composition of the amino acids absorbed cannot be assumed constant.

Disappearance of apparently digestible energy and total amino acids within the large intestine for the three diets confirmed the increased contribution of this section of the tract in the digestion of ground and pelleted forages (Thomson *et al.* 1969; Thomson, Prescott & Armstrong, 1969).

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The utilization of chopped and pelleted lucerne by growing lambs. By D. J. THOMSON and S. B. CAMMELL, *Grassland Research Institute, Hurley, Maidenhead, Berkshire*

The utilization of the dietary energy contained in a dried lucerne (*Medicago sativa* var. Chartainvilliers) given either chopped or ground (1·96 mm screen) and pelleted was measured in a comparative slaughter experiment. Growing lambs were given equal amounts of digestible energy in the chopped or pelleted form at each of three planes of nutrition for a period of 100 d. The energy content of the lambs was determined after slaughter and the initial energy content of the lambs was estimated from the regression equation between fasted (18 h) live weight and total body energy derived from a group of twenty-three lambs slaughtered at the beginning of the experiment. $y = 1{\cdot}673\ x - 5{\cdot}874 \pm 7{\cdot}14$, where y = fasted live weight (kg) and x = body energy (Mcal).

The metabolizable energy content of the diets was derived at each plane of nutrition from measured faecal and urinary losses and methane estimated from relationships given by Blaxter & Clapperton (1965).

The metabolizable energy of the dried lucerne was utilized more efficiently when given in the ground pelleted form than in the chopped form ($P < 0{\cdot}01$) (see Fig. 1). The depression in digestibility of the ground pelleted lucerne diet, compared with the chopped form, and the effect of increasing level of feeding, were both small compared with similar measurements on processed grass diets (Thomson & Cammell, unpublished observations). The gross energy of the dried legume was also utilized more efficiently ($P < 0{\cdot}05$) in the pelleted form than the chopped (see Fig. 2).

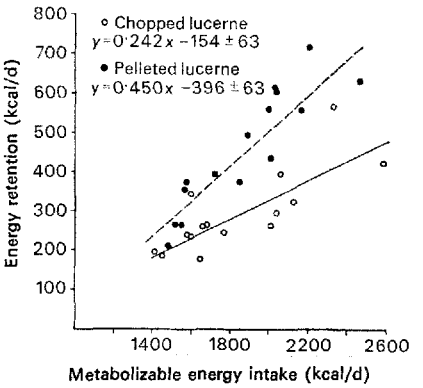

Fig. 1. Energy retention in relation to metabolizable energy intake.

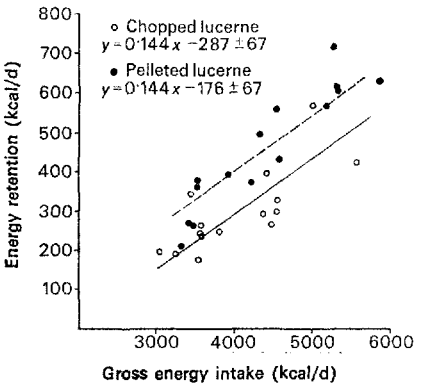

Fig. 2. Energy retention in relation to gross energy intake.

Digestion studies with these same chopped and pelleted lucerne diets (Thomson, Beever, Coehlo da Silva & Armstrong, 1969) have shown that grinding and pelleting depressed the digestion of energy in the fore-stomachs and increased digestion in the small intestine compared with the chopped form. The possible association of

this effect with the increased efficiency of utilization of the gross and metabolizable energy in the ground lucerne diet is being studied.

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Pancreatic secretion in the milk-fed calf. By J. H. TERNOUTH, R. C. SIDDONS* and JOYCE TOOTHILL, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Gorrill, Thomas, Stewart & Morrill (1967) found higher rates of pancreatic fluid secretion as milk-fed calves increased in age from 3 to 21 d, but the amounts of trypsin and protein were maximal at 7 d. Recently, the concentration of amylase in the pancreatic secretion of the calf was observed to be minimal at 7 d and considerably higher at 21 d (Morrill, Stewart, McCormick & Fryer, 1970).

In the present experiments the secretion of pancreatic fluid and enzymes was studied in six Ayrshire calves at three ages, the diet being raw milk (Table 1), and in four Friesian calves at 3 d intervals between 16 and 37 d of age when they were given four milk-substitute diets (Table 2). In each 12 h experiment, the pancreatic secretion was collected continuously from a pancreato-duodenal pouch and returned through a duodenal cannula.

Table 1. *Pancreatic secretion of Ayrshire calves at three ages*

	Enzyme substrate	At mean age (d): 7	24	63	Standard deviation	Significance of difference between means: 7 *v.* 24	24 *v.* 63	7 *v.* 63
No. of experiments		11	10	8				
Volume (ml/12 h)	—	333	467	601	86·3	**	**	***
Trypsin (mg/12 h)	BAEE	146	176	220	87·4	NS	NS	NS
Proteases (g/12 h)†	Casein	1·05	2·84	5·11	1·72	*	NS	**
Amylase (mg/12 h)	Starch	9·36	57·5	240·0	NA	***	***	***

BAEE, benzoyl-arginine ethyl ester; NA, not available, as amylase values are geometric means. NS, $P>0{\cdot}05$. *$P<0{\cdot}05$. **$P<0{\cdot}01$. ***$P<0{\cdot}001$. †As g trypsin activity.

Table 2. *Change of pancreatic secretion with age (d) of Friesian calves*

	Enzyme substrate	Regression	Residual standard deviation	Significance of regression coefficient
Volume (ml/12 h)	—	116·9 +6·41 (±1·12)d	4·31	***
Trypsin (mg/12 h)	BAEE	172·2 +3·54 (±1·81)d	6·95	NS
Proteases (g/12 h)†	Casein	7·79−0·073(±0·040)d	1·55	NS
Amylase (mg/12 h)	Starch	−7·15+0·972(±0·185)d	7·11	***
Lipase (kU/12 h)	Olive oil	−4·73+0·980(±0·177)d	6·89	***
Ribonuclease (mg/12 h)	Ribonucleic acid	3·30+1·09 (±0·16)d	6·08	***

BAEE, benzoyl-arginine ethyl ester. NS, $P>0{\cdot}05$. ***$P<0{\cdot}001$. †As g trypsin activity.

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The increase in volume of pancreatic fluid with age agrees with the work cited above but in the present studies the rates of secretion were higher at all ages. This latter finding cannot be explained by the secretion of the duodenal pouch. Proteolytic enzymes appeared to be secreted at relatively high rates within the first few days of life and subsequent increases were small, whereas the quantities of non-proteolytic enzymes secreted increased rapidly with age.

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Digestion and absorption of lactose by the pre-ruminant calf. By N. B. COOMBE and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Absorption of lactose from the small intestine is achieved by the action of lactase (associated with the brush border of the gut mucosa) splitting the disaccharide into glucose and galactose and the subsequent active absorption of these products. Although some accumulation of glucose and galactose in the gut contents has been reported after infusion of lactose into the human small intestine (Gray & Santiago, 1966), it appears to be generally accepted that, for several species, including man, the rate of lactose absorption is normally limited by the hydrolysis step (Dahlqvist & Thomson, 1964; Gray, 1970). Our investigations suggest, however, that this is not so for the calf.

Two bull calves aged 1 week and 8 weeks, reared previously on only cow's milk, were given a liquid synthetic feed containing lactose as the only carbohydrate. They were slaughtered 3 h after feeding by intravenous injection of an overdose of pentobarbitone sodium. The small intestine and abomasum were exposed, tied off at numerous points and then removed as quickly as possible (about 15 min). The contents of the abomasum and of segments of small intestine, each comprising one-eighth of the total length, were collected separately. Some free glucose and greater amounts of free galactose (up to 150 mmol) were found in the duodenal and jejunal contents, suggesting that lactose hydrolysis occurred more rapidly than absorption of the products. It seems unlikely that post-mortem changes could lead to the liberation of unequal amounts of glucose and galactose, but to eliminate this possibility calves, aged 8–12 weeks, fistulated at two sites (about 200–400 cm apart) in the duodenum and jejunum, were used. Lactose solutions, from 10–280 mM, made isotonic to blood with Na_2SO_4 were infused into the proximal cannula at about 500 ml/h (approximately the rate of passage of digesta). When a steady state was reached, samples from the distal cannula contained free glucose and galactose representing about 35 and 40% respectively of the infused lactose. Incubation in vitro of lactose with digesta from the distal cannula led to no liberation of monosaccharides.

These results suggest strongly that in the pre-ruminant calf lactose is rapidly hydrolysed by the mucosal enzymes to monosaccharides, most of which diffuse back into the lumen to be absorbed lower down the gut.

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Free and conjugated folates in the livers of kittens. By S. J. G. AMYES, MARGARITA E. MONK-JONES, PHYLLIDA M. ROBERTS, PATRICIA P. SCOTT and B. S. SURI, *Department of Physiology, Royal Free Hospital School of Medicine, London WC1*

Folates are present in tissues either as pteroylmonoglutamates or as conjugates containing further, γ-linked, glutamyl residues. In animal tissues the heptaglutamate is the predominant form.

In view of the suggestion by Rabinowicz (1960) that the conjugated folates are the coenzymatically active forms, in vivo, the livers of forty-three kittens aged from 0 to 32 d have been assayed for free and conjugated folate activity. The kittens used were divided into two groups; group 1 was composed of twenty-two kittens born to queens fed on a diet of cooked horse meat supplemented with a mineral and vitamin mixture, (Beta No. 8 TE: Cooper Nutrition Products Ltd, Ilford, Essex) and group 2 comprised twenty-one animals born to queens fed on a mixed diet consisting of tinned and dry cat foods and cooked fish.

Folates in the livers were determined microbiologically using *Lactobacillus casei* as the assay organism. Folate activity was assayed both before and after treatment of tissue extracts with chick pancreas conjugase. This enzyme cleaves the higher conjugates, making them available to *L. casei*. The levels of free folates, consisting of pteroyl mono-, di- and tri-glutamates, and levels of the higher conjugates were calculated. Ascorbic acid was added to all tissue extracts to protect reduced folates against oxidation.

The results for free folate activity reveal a clear division between the groups. All kittens from group 2 had between 11 and 27 μg folate activity in their livers whereas twenty-one out of twenty-two animals from group 1 had between 3 and 10 μg folate activity in their livers (the liver of the twenty-second animal contained 13 μg folate activity). The weights of free folates per liver did not increase with increasing liver weight.

In contrast, the weight of conjugated folates increased linearly with increases in liver weight (and age). Regression lines calculated for each group showed a highly significant correlation ($P<0.001$) but the slopes differed ($P<0.001$). The livers of kittens from group 2 (mixed diet) contained higher concentrations of folate ($P<0.001$) than kittens from group 1 (supplemented horse meat diet). This suggests that in young kittens conjugates may be the metabolically active forms of

folate. Free folate levels were found to be more variable and may be influenced by a number of factors including maternal diet.

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The effect of a glucose syrup drink on plasma triglyceride concentrations after a high-fat meal and a low-fat meal. By L. F. GREEN, T. L. C. DALE, M. A. FORD and R. BAGLEY, *Research and Development Department, Beecham Products, Great West Road, Brentford, Middlesex*

A fall in plasma triglyceride concentration after the intravenous administration of glucose has been reported (Perry & Corbett, 1964) and a similar effect has been demonstrated when glucose is included in a meal (Albrink, Fitzgerald & Man, 1958). It was of interest to confirm whether glucose syrup (a partial hydrolysate of maize starch) had similar effects to glucose on postprandial triglyceridaemia.

Eight apparently healthy men, after an 11 h fast, ate a high-fat meal on two occasions separated by at least a week, during which either a carbonated glucose syrup drink (equivalent to 79 g dextrose monohydrate) or carbonated water was served during the meals. Plasma triglyceride concentrations were determined in the fasting state and during a period of 5 h subsequent to the meals. A comparable experiment was carried out using the same subjects, but eating low-fat meals.

After the low-fat meals, there was no significant difference in triglyceridaemia whether glucose syrup or carbonated water was drunk; however, after the high-fat meals, the concentration of plasma triglycerides was significantly lower when glucose syrup was drunk than when carbonated water was the beverage.

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Serum glucose and fructose concentrations after sucrose meals in atherosclerosis. By I. MACDONALD and L. J. TURNER, *Departments of Physiology and Surgery, Guy's Hospital Medical School London SE1*

Studies of the concentrations of serum fructose and glucose after a sucrose meal (2 g/kg body-weight) were carried out in six men (mean age=58 years) whose atherosclerosis had necessitated surgery. Comparable studies were carried out in six men of the same age group who had had herniorrhaphy.

The results showed that in patients with arterial disease the fructose concentration of the serum rose significantly more than in the controls. The atherosclerotic group had higher fasting serum glucose concentrations than the controls, but the extent of the increase in the glucose concentration was similar in both groups after the ingestion of the sucrose.

We wish to thank the patients and Mr F. Ellis, their surgeon, for their co-operation.

The role of body fat, accumulated during pregnancy, in lactation in the rat.

By D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, London W8*

At parturition, a rat maintained throughout pregnancy on a high-protein diet shows no gain in carcass protein, but has increased her carcass fat by more than 40% (Naismith, 1966). In the present study, the role of this fat reserve in lactation was investigated.

Rats with a mean weight of 215 g were mated, and fed on a diet containing 25% casein. From those producing litters of eleven to thirteen pups, three groups of eight were randomly selected. One group was killed on the 2nd day *post partum* (P+2). A second group was maintained on the same diet, and was killed on the 16th day *post partum* (P+16), and the third group was given a diet containing 11% casein from the 2nd to the 16th day of lactation. The carcasses were analysed for fat and protein. All litters were reduced to eight pups, which were weighed at the beginning and end of the lactation period. Food consumption of the dams was measured.

In a second experiment of identical design, rats weighing 200 g before mating were fed, during lactation, on a diet containing 25% casein supplemented with methionine. Carcass composition was again compared with that of controls killed on the 2nd day of lactation. The results of the analyses are recorded in the table.

Diet	Time of killing	Carcass composition		Gain in weight of individual pups (g)
		Fat (g)	Protein (g)	
25% casein	P+2	40·7	41·5	—
11% casein	P+16	15·8	37·4	12·5
25% casein	P+16	13·4	41·4	19·5
25% casein	P+2	32·5	39·2	—
25% casein+methionine	P+16	13·0	42·2	24·3

The gain in weight of the pups, an index of milk yield, was related direct to the protein value of the maternal diet.

The daily food intake of the rats fed on the low-protein diet was constant throughout lactation, whereas the dams receiving the high-protein diets had more than doubled their daily food intakes by the end of lactation.

The rats fed on the 25% casein diet lost no body protein, but lost almost 70% of their body fat. An improvement in the protein value of the diet permitted a significant increase in carcass protein, but fat was again catabolized. Thus, on high-protein diets, optimum use is made of dietary protein for the synthesis of milk protein, the additional energy for milk formation being supplied from oxidation of the fat accumulated during pregnancy.

In contrast, rats fed on the low-protein diet contributed 10% of their body protein, in addition to calories from body fat, towards the synthesis of a much reduced volume of milk.

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The stability of vitamin C in machine-peeled potatoes. By L. ZARNEGAR and A. E. BENDER, *Department of Nutrition, Queen Elizabeth College, London W8*

It has been reported that machine-peeled potatoes, subjected to prolonged soaking in water, lose considerable amounts of vitamin C compared with hand-peeled potatoes, namely 46% compared with 9% (Platt, Eddy & Pellett, 1963). The importance of potatoes as a source of vitamin C and the widespread criticism of institutional cooking make this report a matter of considerable practical importance. Before attempting to explain why machine peeling should have so destructive an effect, the experiment was repeated on a somewhat larger scale. (The original finding was based on a single sample.)

Twelve potatoes (King Edward variety) from the same sack were used for each group of analyses in the following treatments: (1) whole, unpeeled, raw potatoes; (2) hand-peeled potatoes analysed immediately; (3) as (2) soaked in tap-water for 20 h; (4) as (2) soaked in water for 40 h; (5) peeled for 6 min in a Hobart mechanical peeler, fully loaded, and analysed immediately; (6) as (5) soaked in water for 24 h; (7) as (5) only half filling the machine to increase mechanical treatment; (8) as (7) soaked for 24 h.

Results were as shown in the table.

Table 1. *Vitamin C in raw potatoes (mg/100 g); twelve samples in each group*

Treatment		Average	Range	SD	Loss	Significance *P* value
Unpeeled	1	12·4	9·0–15·5	1·8		
Hand-peeled	2 Analysed immediately	12·8	9·7–16·1	1·8		
	3 Soaked for 20 h	11·9	8·8–18·9	2·6	7%	>0·05 3 *v.* 2
	4 Soaked for 40 h	11·5	9·7–12·6	0·8	10%	<0·05>0·02 4 *v.* 2
Machine-peeled 6 min; fully loaded	5 Analysed immediately	12·6	8·6–14·0	1·3		
	6 Soaked for 24 h	10·1	8·1–11·8	1·0	20%	<0·001 6 *v.* 5
Machine peeled 6 min; half loaded	7 Analysed immediately	11·7	8·3–14·6	1·7		
	8 Soaked for 24 h	10·3	6·4–11·2	1·4	12%	<0·05>0·02 8 *v.* 7

The finding that the loss of vitamin C in (8) was smaller and less significant than in (6) was possibly due to the analysis of different groups of twelve potatoes. The results indicate that mechanical peeling and soaking may be no more destructive than hand-peeling and soaking, but certainly far less destructive than earlier reports suggest.

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