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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Ninety-seventh Meeting of the Nutrition Society was held in the Allen Lecture Theatre, Atkins Building (Chemistry Entrance), Queen Elizabeth College, Campden Hill, London W8 7AH, on Friday, 1 *October,* 1976, *when the following papers were read:*

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Arteriovenous difference studies on the lactating rabbit mammary gland. By C. S. JONES and D. S. PARKER, *Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading*

Rabbit milk fat contains a high proportion of octanoic (C8:0) and decanoic (C10:0) fatty acids, over 70 molar per cent (Smith, Watts & Dils, 1968) which are synthesized within the mammary gland. The rabbit has a circulating blood glucose concentration comparable to that of other non-ruminant species, although it has been postulated that acetate is the major precursor for these medium chain fatty acids (Popjak, Hunter & French, 1953; Carey & Dils, 1972). Our initial investigation was designed to establish cannulae in the lactating doe in order to determine which metabolites were being utilized by the mammary gland.

The carotid artery and lateral thoracic vein of New Zealand White (NZW) does approximately 14 d post partum were cannulated and the does returned to their young. After allowing at least 24 h to recover from surgery, simultaneous blood samples were obtained from the cannulae at intervals. To ensure that no change in milk composition occurred between the glands on the operated side of the doe and the glands on the other side, milk was collected before surgery and after all blood samples had been taken. The milk samples were assayed for protein, lactose and fat, and the results showed no statistical significant difference between the two sets of glands.

The blood samples were assayed enzymatically for glucose and 3-hydroxybutyrate (3-HBA) and by gas liquid chromatography for acetate. The results in Table 1 show a significant uptake of these metabolites, although it is difficult to quantify this data without consideration of blood flow through the gland.

Table 1. *Concentration of metabolite (mM) in the carotid artery and lateral thoracic vein of NZW does 14 d post partum*

(Mean values with standard errors, number of samples in parenthesis)

		Arterial (A)		Venous (V)		A–V		$\frac{A-V}{A} \times 100$	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Glucose**	(30)	5·68	0·15	4·60	0·14	1·08	0·10	19·01	1·61
Acetate**	(24)	0·44	0·04	0·14	0·01	0·30	0·05	68·18	3·33
3-HBA*	(30)	0·19	0·02	0·11	0·02	0·08	0·01	42·11	3·33

Mean values for arterial and venous samples differ significantly $^{**}P<0.001$, $^{*}P<0.01$.

Comparison of the data shown in Table 1 with that available for other species (Linzell, 1974) indicates that the requirements of the mammary gland of the rabbit resemble those of the ruminant rather than of the non-ruminant animal. The importance of acetate, rather than glucose, as a precursor for milk fat synthesis is in agreement with radioisotope infusion experiments carried out in this laboratory (C. S. Jones, unpublished data).

Carey, E. M. & Dils, R. (1972). *Biochem. J.* **126**, 1005.
Popjak, G., Hunter, G. D. & French, T. H. (1953). *Biochem. J.* **54**, 238.
Smith, S., Watts, R. & Dils, R. (1968). *J. Lipid Res.* **9**, 52.
Linzell, J. L. (1974). In *Lactation* [B. L. Larson and V. R. Smith, editors]. New York and London: Academic Press.

Development of the digestive tract of the rabbit from birth to weaning.

By GÜLDEN ALUS and N. A. EDWARDS (Introduced by D. S. PARKER), *Department of Physiology and Biochemistry, The University of Reading, Reading RG6 2AJ*

Study of the stomach contents of New Zealand White rabbits from birth to 30 d of age indicated the following stages: (1) total dependence on milk up to 10 d; (2) a small consumption of solid food (about 5%) at 15 d; (3) by 20 d coprophagy begins and most ingestion is of solid food; (4) from 25 to 30 d the rabbit consumes only a trace of milk and actively coprophagates. This development was associated with certain anatomical and enzymatic changes of the digestive system.

The growth of the caecum where material for coprophagy is produced was logarithmic being related to age (t, days) by the equation: log caecal wt (g)=0·056t−0·86. Thus caecal growth was relatively slow from birth to 10 d but from 10 to 30 d rapid growth produced a 14 times weight increase. The mass of the caecal contents showed a similar pattern of change. On the other hand the rates of growth of the stomach and small intestine appeared linear from 0 to 30 d being expressed by the equations: stomach wt (g)=0·20t+0·34; intestine wt (g)=0·45t+0·98.

The rise in consumption of solids from 20 to 30 d was accompanied by an increase in the levels of intestinal sucrase (glucosidosucrase, *EC* 3.2.1.20) and maltase (α-glucosidase, *EC* 3.2.1.20) with activities (per g protein) rising by some 27 and 14 times respectively in both the ileum and jejunum. Intestinal lactase (β-galactosidase, *EC* 3.2.1.23) decreased gradually from birth to 30 d.

The total levels of pancreatic trypsin and chymotrypsin showed linear increases of 8·8 and 7·0 times respectively over the 30 d. This was due to a rise in the mass of the pancreas rather than to a change in the concentration of the enzymes in the organ. By contrast, the pancreatic amylase rose slowly from 0 to 15 d, and then increased by 60 times over the following 15 d.

These results indicate that, as solid ingesta replace milk as the main dietary components of the rabbit, the enzymes associated with the digestion of carbohydrates other than lactose increase rapidly in quantity. At the same time extensive enlargement of the caecum occurs and caecal fermentation and coprophagy begin.

Digestibility and coprophagy in the growing rabbit. By A. G. STEPHENS, *Department of Physiology and Biochemistry, The University of Reading, Reading RG6 2AJ*

The prevention of coprophagy has been shown to decrease dry matter (DM) and protein digestibility in rabbits (Thacker & Brandt, 1955). The following investigation was carried out to confirm these effects in rabbits fed a commercial laboratory ration.

New Zealand White male rabbits, aged 6–8 weeks, live weight 1·0–1·5 kg were used. They were caged individually and fed *ad lib.* a pelleted ration (Diet 18, E. Dixon & Sons Ltd, Ware), having the following composition (g/kg): DM, 911; ash, 103; ether extract, 54; crude protein (N×6·25), 266; crude fibre, 124; nitrogen-free extract, 364; true protein, 195 and acid detergent fibre, 170.

Rabbits were given this diet for a pre-trial period of at least 7 d. Food intake and faecal output of individual rabbits were then monitored over a period of 8–10 d. Coprophagy was prevented by the use of plastic collars.

The results are summarised in the table:

	Food intake (g/d)	Weight gain (g/d)	Dry matter	Apparent digestibility coefficients: Crude fibre	Acid detergent fibre	Crude protein	True protein
Coprophagy allowed (14 replicates)	115·8± 5·1	40·1± 2·2	0·608± 0·007	0·028± 0·010	0·157± 0·017	0·765± 0·007	0·713± 0·010
Coprophagy prevented (6 replicates)	122·9± 8·3	23·9± 5·2	0·582± 0·019	0·016± 0·014	0·121± 0·032	0·686± 0·024	0·666± 0·025

(Values are means ±SE).

Preventing coprophagy did not significantly affect food intake, but it did decrease the rate of live weight gain ($P<0·05$). The apparent digestibility coefficients of DM, acid detergent fibre, crude fibre and true protein showed no significant difference, although that for the crude protein fraction was significantly lowered when coprophagy was prevented ($P<0·01$). These data indicate that coprophagy is beneficial to the rabbit and increases the digestibility of N in the diet.

In all rabbits very low digestibilities of crude fibre were observed confirming the findings reported by Davidson & Spreadbury (1975). However, the digestibility of the acid detergent fibre fraction was much higher. Van Soest & McQueen (1974) have described the limitations of the crude fibre method as a means of determining insoluble carbohydrates and lignin. The low levels of digestion of crude fibre are probably the result of the failure of the method to quantify fibre as a constant proportion of the insoluble carbohydrates and lignin present.

Davidson, J. & Spreadbury, D. (1975). *Proc. Nutr. Soc.* **34**, 75.
Thacker, E. J. & Brandt, C. S. (1955). *J. Nutr.* **55**, 375.
Van Soest, P. J. & McQueen, R. W. (1973). *Proc. Nutr. Soc.* **32**, 123.

The metabolism of lactic acid in the large intestine of the rabbit. By D. S. PARKER and A. J. MOULD, *Department of Physiology and Biochemistry, The University of Reading, Reading RG6 2AJ*

Bacterial fermentation in the large intestine of mammalian species may result in the production of lactic acid and the concentration of this acid will depend upon the balance between lactate-producing and lactate-utilizing organisms present in the digesta. To investigate the production and possible absorption of lactic acid in the large intestine of the rabbit, cannulae were established in the carotid artery and the anterior mesenteric vein of three animals of the New Zealand White strain. Samples of blood were taken from both cannulae over a period of several days, and after sacrificing the animals samples of caecal digesta were obtained. All samples were analysed enzymatically for both L(+)- and D(−)-isomers of lactic acid. The results obtained were:

	Carotid artery (mmol/l)	Anterior mesenteric vein (mmol/l)	Caecal contents (μmol/g wet wt)
L(+)-Lactic acid	1·096±0·136 (16)*	2·019±0·215 (18)*	0·308±0·006 (9)
D(−)-Lactic acid	0·094±0·025 (6)	0·056±0·013 (6)	ND

(Mean values with standard errors, number of samples in parenthesis)

*Sample means differ significantly $P<0·01$.

The concentration of lactic acid found in the caecum was similar to that reported previously (Alexander & Davies, 1963) and although the concentrations in the two series of blood samples were within the expected range for the rabbit, we obtained significantly higher values ($P<0·01$) in venous blood when compared with arterial samples. The source of the D(−)-isomer in the blood was thought to be bacterial action in the stomach which results in the production of a greater proportion of D(−)- than L(+)-lactic acid (Hornicke & Mackiewicz, 1976), there being no detectable D(−)-isomer in the caecal digesta.

The metabolism of lactic acid in caecal contents was followed using [1−^{14}C] and [2−^{14}C] DL-sodium lactate. Samples of caecal dialysate (Parker & McMillan, 1976) were obtained at intervals after a single injection of isotope (25 μCi) into the caecum and the volatile fatty acid (VFA) fraction was analysed by radio–gas–liquid–chromatography. The results showed that within 10 min of the injection of isotope 85% of the radioactivity recovered was associated with the VFA, thus indicating rapid metabolism of lactic acid by the microbial population. The distribution of the radioactivity between the individual VFA confirmed that lactic acid was metabolised via pyruvic acid to two carbon units, and that the acrylate pathway of propionic acid production might also be present.

This work was supported by the Science Research Council.

Alexander, F. & Davies, M. E. (1963). *J. Comp. Path.* **73**, 1.
Hornicke, H. & Mackiewicz, A. (1976). *Proceedings of the 1st International Rabbit Congress*, Dijon (France).
Parker, D. S. & McMillan, R. T. (1976). *Br. J. Nutr.* **35**, 365.

Estimation of microbial nitrogen compounds entering the ruminant duodenum using different reference components including a ^{32}P label. By R. H. SMITH, PATRICIA E. LEWIS and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Dietary nucleic acids entering the rumen are rapidly degraded so that it is probable that RNA entering the duodenum of the ruminant is largely of microbial origin (McAllan & Smith, 1973). Thus measuring the amount of RNA in duodenal digesta may provide a means of assessing the contribution of microbial components at that site, although some inaccuracy may be introduced by small amounts of dietary RNA surviving. To examine the extent of this possible interference, young steers cannulated in the rumen and proximal duodenum were given an appropriate diet for at least 20 d, supplemented for part of the time with 0·5 mCi ^{32}P as inorganic phosphate/d. Experiments relating the composition of samples of rumen bacteria with that of corresponding samples of duodenal contents were carried out after 10–12 d of ^{32}P supplementation. Ratios of bacterial-nitrogen to total-nitrogen in duodenal contents were calculated using total RNA, ^{32}P-labelled RNA and α, ε-diaminopimelic acid (DAP) respectively as reference materials. Results (Table 1) based on total RNA were generally the highest indicating the probable presence of a small amount of dietary RNA at the duodenum. It is, however, possible that at least some of the difference was due to the presence of protozoal compounds in duodenal digesta which would be broadly included in an estimate based on total RNA, excluded from one based on DAP (McAllan & Smith, 1974) and included to an uncertain extent (depending upon how much preformed dietary nucleic acid was incorporated by the protozoa) in an estimate based on ^{32}P-labelled RNA.

Table 1. *Ratios of bacterial-N to total-N in duodenal digesta estimated by different methods*

(Each value is a mean with standard error for two steers)

	Method					
	RNA		[^{32}P]RNA		DAP	
Diet	Mean	SE	Mean	SE	Mean	SE
Flaked maize+hay	0·71	0·09	0·59	0·09	0·55	0·01
Flaked maize+hay+ground nut meal	0·72	0·27	0·57	0·13	0·55	0·03
Flaked maize+hay+fishmeal	0·59	0·11	0·50	0·04	0·48	0·02
Dried grass	0·46	0·05	0·50	0·01	0·57	0·11

In some experiments, estimates of bacterial-N/total-N at the duodenum were based upon the readily determined ^{32}P activity in a residue obtained by extracting bacteria or digesta with fat solvents and aqueous trichloracetic acid. Mean estimates (6 experiments, 3 calves) (0·53, SE±0·03) were not significantly different from those based upon ^{32}P-labelled RNA (0·50, SE±0·04). The technique promises to be of practical value.

McAllan, A. B. & Smith, R. H. (1973). *Br. J. Nutr.* **29**, 331.
McAllan, A. B. & Smith, R. H. (1974). *Proc. Nutr. Soc.* **33**, 41A.

Use of ^{35}S-incorporation for the measurement of microbial protein in ruminant digesta. By J. C. MATHERS and E. L. MILLER, *Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX*

Estimation of bacterial protein synthesis in the rumen of sheep by ^{35}S-incorporation was first reported by Roberts & Miller (1969). Their method was applicable to measurements made in the rumen and has been developed for use in estimation of microbial protein in abomasal or duodenal digesta.

Microbial protein synthesized in the rumen was labelled with ^{35}S by intra-ruminal infusion of $Na_2{}^{35}SO_4$ (150 μCi/d in 2000 ml water) for 5 d, and a microbial fraction was isolated from abomasal digesta (days 3–5) using differential centrifugation at 20 000*g* for 20 min after 1000*g* for 1 min. Freeze-dried samples of this fraction (150 mg) and of abomasal digesta (300 mg) were oxidized with performic acid (20 ml for 16 h at 4°) to convert inorganic ^{35}S to $^{35}SO_4$ and cystine and methionine to the more stable cysteic acid and methionine sulphone. Oxidation was stopped by the addition of 3 ml hydrogen bromide and the flask contents were rotary evaporated to near dryness prior to hydrolysis under reflux with 20 ml 6 M-HCl for 22 h. The hydrolysate was filtered, rotary evaporated to near dryness, transferred using 3×2 ml distilled water to a centrifuge tube containing 1 ml saturated $BaCl_2$ and $Ba^{35}SO_4$ removed by centrifugation. The ratio of counts to N was determined on the supernatant and corrected for the ammonia-N of the original freeze-dried sample to give a ratio of ^{35}S to non-ammonia-N (NAN). The proportion of microbial NAN in the digesta NAN was calculated as ^{35}S:NAN [digesta]÷^{35}S:NAN [microbial].

Known mixtures of labelled abomasal digesta and sunflower seed meal were prepared in vitro and assayed by the above procedure. Good estimates of the proportions of microbial and sunflower seed meal NAN in the mixtures were obtained.

For two sheep given 960 g grass nuts/d in 12 feeds, 45·8 and 50·5% of the NAN in abomasal digesta were determined as being of microbial origin. Total NAN flow to the abomasum was measured using chromic oxide as a marker and assuming 2 g/d endogenous NAN flow to the abomasum, 47·9 and 49·8% of the grass N were calculated to be degraded in the rumen of the two sheep.

Roberts, S. A. & Miller, E. L. (1969). *Proc. Nutr. Soc.* **28**, 32A.

Exchanges of digesta components in different compartments of the stomach of the young steer. By B. M. EDRISE, R. H. SMITH and H. L. BUTTLE, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Oyaert & Bouckaert (1961) studied omasal function by collecting digesta flowing at the omasal/abomasal orifice in sheep. Engelhardt & Hauffe (1975), using a flexible plastic sleeve, sutured at this site, with access through an abomasal cannula, have exploited this approach with notable success.

A suitably cannulated steer was equipped with a similar sleeve and given a diet consisting of dried grass and flaked maize (3:2) with mineral and vitamin supplements and polyethylene glycol (PEG) in two feeds/d at 09.00 and 17.00 hours. In four experiments samples were taken before the morning feed and at 2, 4 and 6 h after it, from the rumen, reticulum, omasal outflow and abomasum. For the 2 h samples, concentrations of certain components in rumen contents and estimates of proportions of these components and water which were recovered at other sites relative to PEG (Component/PEG at chosen site × PEG/Component in rumen) were:

Digesta component	Concentration in rumen contents (mmol/l)		Proportions of amounts in the rumen					
			Reticulum		Omasal outflow		Abomasum	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Water	—	—	1·03	0·12	0·53	0·03	1·05	0·05
Sodium	109·2	3·8	0·80	0·05	0·31	0·02	0·52	0·04
Potassium	42·1	3·7	0·96	0·08	0·89	0·09	1·02	0·08
Chloride	10·7	0·4	0·83	0·07	2·97	0·24	9·89	0·30
Acetate	63·2	2·0	0·71	0·07	0·27	0·01	0·21	0·02
Propionate	22·2	0·9	0·72	0·09	0·25	0·01	0·19	0·01
Butyrate	14·6	1·4	0·62	0·09	0·19	0·01	0·14	0·01
Ammonia	0·20	0·08	0·52	0·19	5·44	1·68	12·11	1·47

Different concentrations and pH values were found at different times after feeding but patterns of exchange of components between sites were generally similar and pH values in the reticulum were consistently higher (by about 0·3–0·6 pH units) than in rumen or omasal outflow. Proportions of water and sodium absorbed between reticulum and omasal outflow (about 0·5 and 0·6 respectively) were much greater than in the sheep or goat (Engelhardt & Hauffe, 1975) but absorption of volatile fatty acids (VFA) was only slightly greater. Further absorption of VFA occurred in the abomasum but other components showed a net addition at this site. As for sheep or goat marked addition of chloride occurred in the omasum as well as the abomasum. Net addition of ammonia occurred in both omasum and abomasum, presumably because concentrations were very low (Smith & McAllan, 1971).

Engelhardt, W. v. & Hauffe, R. (1975). In *Digestion and Metabolism in the Ruminant*, p. 216 [I. W. McDonald and A. C. I. Warner, editors]. Armidale: University of New England.

Oyaert, W. & Bouckaert, J. H. (1961). *Res. vet. Sci.* **2**, 41.

Smith, R. H. & McAllan, A. B. (1971). *Br. J. Nutr.* **25**, 181.

The effects of dietary factors on acid-induced inhibition of reticulo-ruminal motility in sheep. By P. K. UPTON, J. P. RYAN and B. F. LEEK, *Department of Veterinary Physiology and Biochemistry, Veterinary College of Ireland, Dublin 4*

Clinical ruminal acidosis, with its associated reticulo-ruminal stasis, is likely to arise from engorgement of concentrate feeds or of acid treated feedstuffs.

The amount of 2–4 M acids infused slowly (40–80 mmol/min) through the ruminal cannulae of 6 sheep, on a comparable dietary regime (IL), which abolished reticulo-ruminal motility was:

Acid:	Hydrochloric (IL)	Lactic (IL)	Formic (IL)	Acetic (IL)	Propionic (IL)	Butyric (IL)	SE
At abolition; amount infused (mmol)	563	1570	912	730	1043	543	231
Rumen fluid pH	4·71	3·99	4·55	5·26	5·05	5·02	0·40

Acid:	Hydrochloric			Acetic			Lactic			
	(HL)	(IL)	(LL)	(HL)	(IL)	(LL)	(HL)	(IL)	(LL)	SE
At abolition; amount infused (mmol)	1615	963	316	1876	1216	764	2631	1996	1084	309
Rumen fluid pH	3·25	4·20	5·35	4·84	5·15	5·42	4·50	4·37	4·79	0·40

HL Sheep were given 1 kg sheep pellets 18 h before the infusion and 0·5 kg sheep pellets within 0·5 h of the start of the infusion of the acid.

IL Sheep were fasted overnight and given 0·5 kg sheep pellets within 0·5 h of the start of the infusion of the acid.

LL Sheep were fasted overnight and given no dietary pretreatment prior to the infusion of the acid.

The results show that although lactic acid caused the greatest decrease in the rumen fluid pH, butyric, acetic and hydrochloric acids were significantly ($P<0·05$) more potent in inhibiting motility than lactic acid, with formic and propionic acids being of an intermediate potency.

The influence on motility of a high (HL), intermediate (IL) and low level (LL) of feeding in the 18 h prior to the infusion (40–80 mmol/min) of hydrochloric, acetic and lactic acids into the rumens of 9 sheep was also examined. The capacity of the acids to inhibit motility was significantly influenced ($P<0·001$) by the dietary pretreatments with the inhibitory effects of the acids being inversely related to the amounts of food consumed prior to the infusion, while the rumen fluid pH in the case of the 3 acids used was higher at the point of abolition on the low level of feeding compared with the high level.

It is concluded (1) that the differing inhibitory properties of the acids in this study are not directly related to their capacity to induce a reduction in the pH of the ruminal contents, which accords with the results of Leek, Ryan & Upton (1976), and (2) that the amount of diet consumed and the proximity to feeding can exert a substantial influence on the inhibitory properties of ruminal acidification.

Leek, B. F., Ryan, J. P. & Upton, P. K. (1976). *J. Physiol., Lond.* (In the Press.)

The effect of the inclusion of sodium bicarbonate in concentrate diets for artificially-reared lambs. By P. CORCUERA, P. D. PENNING and T. T. TREACHER, *Grassland Research Institute, Hurley, Maidenhead SL6 5LR*

Although the response of the adult ruminant to the inclusion of buffer salts in the diet is very variable (Diven, 1975), Kellaway, Grant & Chudleigh (1973) suggest that the addition of buffer salts to the diet causes a large increase in intake in the first weeks after early weaning of calves. If a similar response occurs in the lamb, buffer salts might be used to reduce the check in growth that occurs, as a result of low solid-food intake, when artificially-reared lambs are weaned before 42 d of age.

The effect of including sodium bicarbonate in a concentrate diet based on barley was assessed in a factorial design using six lambs per treatment. Starting at 1 d of age milk substitute was fed to give gross energy intakes of 2·25 (L) or 7·50 (H) MJ/d for 23 or 33 d. Pelleted diets containing 0, 30 or 60 g $NaHCO_3$/kg fresh diet were offered *ad lib.* together with chopped barley straw until slaughter at 35 kg live weight: the digestibility of the dietary organic matter was 0·716, 0·745 and 0·770±0·0065 respectively.

The intakes of concentrate before weaning were low and influenced by milk intake, (L) 38 and (H) 19 g/d, and weaning age, (23 d) 15 and (33 d) 42 g/d but not by $NaHCO_3$ content of the diet.

After weaning the intake of straw, although significantly influenced by pre-weaning treatment and $NaHCO_3$ content of the diet, was never greater than 6% of the concentrate intake. The inclusion of $NaHCO_3$ increased the daily intake of concentrate (CDMI), the rate of live weight gain (LWG), and the efficiency of food conversion (FCE). These responses were greater at the low level of milk feeding. The time taken to reach slaughter weight (DTS) and the total concentrate intake (TCDMI) were reduced by the inclusion of $NaHCO_3$.

Milk substitute allowance:	L			H			
Level of $NaHCO_3$ inclusion (g):	0	30	60	0	30	60	SE of mean
CDMI (g/d)	771^a	955^b	911^b	917^b	936^b	1033^c	25·7
LWG (g/d)	207^a	321^c	320^c	255^b	302^c	336^c	9·5
FCE* (g/100 g)	$27·2^a$	$33·7^b$	$35·2^b$	$27·9^a$	$32·2^b$	$32·6^b$	0·72
DTS (days)	166^a	119^b	124^b	119^b	107^c	96^d	3·3
TCDMI (kg)	108^a	87^b	85^b	82^b	73^c	70^c	2·3

Means with different superscript in the same line are significantly different ($P<0·05$)
*FCE=g LWG/100 g CDMI.

At slaughter the pH and total VFA concentration of the rumen contents of the lambs on the $NaHCO_3$ diets were significantly higher.

Diven, R. H. (1975). *Feedstuffs* **47**, (32), 23.
Kellaway, R. C., Grant, T. & Chudleigh, J. W. (1973). *Aust. J. Exp. Agric. & Anim. Husb.* **13**, 225.

_N_-paraffin-grown yeast in chick diets: retention of phosphorus. By T. OGUNTONA, R. J. NEALE and D. LEWIS, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

A very low calcium to phosphorus (Ca:P) ratio is characteristic of yeast (Kihlberg, 1972) necessitating the supplementation of yeast-rich chick diets with high levels of easily assimilable inorganic calcium. The extent to which the yeast P is available to the chick, however, is unknown. In earlier work (unpublished observations) chicks fed a Ca-supplemented 300 g/kg *n*-paraffin-grown yeast diet containing P in excess of the recommended level (ARC, 1975) showed a positive growth response to supplementary inorganic P. It was, therefore, speculated that the yeast P was probably poorly available to the growing chicks when the yeast was included at high levels in practical-type diets.

In the present study, the growth performance and P retention by chicks from a 300 g/kg *n*-paraffin-grown yeast diet supplemented with graded amounts of phosphorus has been measured.

Five diets were used. The control diet (diet 1) was based on soya bean and fishmeal. Diets 2–5 contained 300 g/kg *n*-paraffin-grown yeast replacing the soya–fish fraction in diet 1. Inorganic calcium phosphate was used to achieve an additional P supplementation of 1·5, 2·0 and 2·5 g/kg in diets 3, 4 and 5 respectively and maintaining a near-constant Ca content.

Each diet was allocated to four groups of three male Cobb chicks for 14 d. The results are shown in Table 1.

Table 1. *Food conversion ratio, weight gain, phosphorus retention and tibia ash of chicks fed P-supplemented n-paraffin-grown yeast diets*

(Values are means of four replicate groups of three chicks)

Diet	Supplementary P (g/kg)	Total dietary P (g/kg)	Bodyweight gain (g/d)	Food conversion ratio*	P retention (% P ingested)	Tibia ash (% dry wt)
1	—	10·0	$23·5^{a}$	$0·647^{a}$	$72·0^{a}$	$48·1^{a}$
2	—	9·5	$22·1^{a}$	$0·606^{b}$	$46·8^{b}$	$44·9^{b}$
3	1·50	11·0	$22·8^{a}$	$0·608^{b}$	$55·1^{c}$	$45·9^{bc}$
4	2·00	11·5	$23·7^{a}$	$0·636^{ac}$	$60·4^{d}$	$46·5^{c}$
5	2·50	12·0	$23·5^{a}$	$0·633^{c}$	$58·5^{d}$	$46·3^{c}$

*g wt gain:g food intake.

Mean values without a common superscript letter are significantly different ($P<0·05$)

The results show that in high yeast diets additional P over and above the recommended levels improved food conversion efficiency, P retention and tibia ash compared to an unsupplemented high yeast diet (2). The maximum response occurred with diet 4, extra P producing no further improvement. The results suggest that the phosphorus in *n*-paraffin yeast is not highly available to the chick.

We acknowledge the support of BP Proteins in carrying out this work.

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High and low erucic acid rapeseed oil in the diet of lactating rats.

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It is generally accepted that inclusion of high erucic acid rapeseed oil (HERSO) at 15% w/w or more in the diet of the weanling rat causes a rapid but transient increase in lipid content of the heart and reduces growth rate, food intake and efficiency of energy utilization, when compared to either low erucic acid rapeseed oil (LERSO) or other control oils (Vles, 1975).

This experiment was to investigate whether similar effects occurred in the sucking rat. Rats, 6 per treatment, of CFY strain (Remote Sprague Dawley, Anglia Laboratory Animals, Huntingdon) were given diets, containing either 21% w/w HERSO (50·8% w/w erucic acid) or LERSO (5·5% w/w erucic acid) or a corn oil–beef tallow mixture, 1:1, (CO:BT), or a diet containing 1% w/w corn oil (low fat, LF) with protein, minerals and vitamins at a constant ratio to energy, through late pregnancy and 16 d of lactation. Litters were equalized to 8 pups at 3 d and killed at 16 d of age.

Cardiac fatty acids of 16 d pups were very low on all treatments and were not increased by the rapeseed oil treatments nor was there any difference between HERSO and LERSO treatments even though erucic acid comprised 21% w/w of the rat-milk fatty acids greater than C16, as taken from the stomachs of 16 d pups, of rats given HERSO. Erucic acid was found to be present in cardiac fatty acids in similar proportions to those in the long chain fatty acids of the rat milk.

Weight gains of male and female pups and daily food intake during lactation of the mothers on both the HERSO and LERSO treatments were significantly decreased compared with the CO:BT control. Although the food intake by the mothers was similar for both rapeseed oil treatments, the pups of the HERSO treatment may have had a lower energy intake as evidenced by the smaller (not significant) weight gain, decreased carcass fatty acids and decreased milk in the stomach at 16 d.

	Treatment					Significance of contrasts		
	LF	CO:BT	LERSO	HERSO		1*v*	2*v*	
	1	2	3	4	SEM	2+3+4	3+4	3*v*4
Weight gain of pups: (g/16 d)								
Males	24·0	28·2	22·3	18·5	1·59	NS	••	NS
Females	23·4	27·2	22·4	18·4	1·66	NS	••	NS
Food intake of mothers (g/d)	34·6	32·7	27·8	27·2	1·64	•	•	NS
Milk in stomach (g/pup)	1·54	1·62	1·54	0·87	0·187	NS	NS	•
Cardiac fatty acids of pups (mg/g wet tissue)	14·3	25·4	14·9	20·8	2·66	NS	•	NS
Carcass fatty acids of pups (mg/g wet tissue)	45·6	99·7	102·9	44·6	9·15	••	•	•••

NS, non-significant; • $P<0.05$; •• $P<0.01$; ••• $P<0.001$.

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A DIY human calorimeter for £100. By G. A. BROWN, H. P. BENNETTO, D. S. MILLER, M. RIGBY, M. J. STOCK, and J. L. STIRLING, *Queen Elizabeth College, Campden Hill, London W8 7AH*

The early human calorimeters used elaborate systems for the control of coolant temperatures and massive insulation. Much of our understanding of human energy expenditure dates from this early work, largely one suspects because the task of repeating it appeared too formidable. Recently human gradient-layer calorimeters have been built but the cost of these is prohibitive to most workers, certainly too expensive for teaching purposes. Our calorimeter is cheap because it takes advantage of two factors, modern insulating materials, and coolant water from a rising main which is remarkably constant in temperature over the period required. The controlled temperature of the chamber is maintained by an electrical heating system which is monitored on a domestic watt-hour meter. A plywood chamber covered with polystyrene is constantly cooled using the mains water which circulates through pipes covering the inside walls. The chamber is ventilated by a vacuum cleaner (50 l/min) and the air within the chamber is stirred by two electric fans. Evaporative heat loss is contained within the chamber since the pipes have a temperature below the dew-point; the relative humidity and temperature of the air entering and leaving the chamber are the same.

Heat losses from a human subject (or a calibrating heat source) may be measured in two ways. Method A: the electricity used to maintain the chamber temperature can be measured with and without the subject, the difference being the amount of heat lost by the subject. Method B: the product of the change in temperature (by thermistors) of the coolant water and its flow (by a waterboard meter) give the total heat removed, from which subtraction of the heater energy gives the heat loss of the subject.

The calorimeter as exhibited also functions as a respirometer i.e. an open-circuit indirect calorimeter, by measurement of oxygen consumption. Thus a comparison between heat production (by indirect calorimetry) and heat loss (by direct calorimetry) in man is also possible.

		Heat loss		Heat production		Difference	
	n	(W)	SE	(W)	SE	(W)	SE
	Electrical standard (heater tape rated at 104 W)						
Method A	14	110·7	2·3	118·2	0·6	+ 7·5	2·4
Method B	14	116·1	4·7	118·2	0·6	+ 2·1	4·5
	Butane standard (variable butane torch: range 89–161 W)						
Method A	16	111·2		116·2		+ 5·0	2·7
Method B	16	119·9		116·2		− 3·7	4·1
	Human subject (Method B)						
Pre-prandial	19	57·8	5·8	75·0	0·9	+17·2	5·8
After nutramente (1·67 MJ)	6	86·0	6·7	85·8	2·1	− 0·2	6·9
After alcohol (0·65 MJ)	5	87·4	7·4	80·4	3·9	− 7·0	11·1
After no food (control)	8	68·5	5·3	78·6	2·4	+10·1	5·1

The values given are in watts (J/s) measured over a period of 1 h: to convert to kJ/min multiply by 0·06. All the human values were measured on one subject: the differences shown in the last column do not necessarily represent errors but could indicate changes in thermal balance i.e. changes in body temperature (see Pittet, Gyax & Jéquier, 1974).

An automated apparatus for measuring daily energy expenditure in laboratory animals. By M. BOROUMAND and D. S. MILLER, *Nutrition Department, Queen Elizabeth College, Campden Hill, London W8 7AH*

Whereas many kinds of apparatus for measuring the oxygen consumption of laboratory animals over short periods of time have been described, techniques for measuring daily energy expenditure tend to be elaborate and laborious. These limitations have often required the experimental animal to be restrained or anaesthetised, or required to breathe pure oxygen: thus the measurement is made under abnormal conditions and is inappropriate for monitoring energy balance over long periods of time. The present apparatus is designed to take the habitual cage of the animal to be measured, and hence minimal disturbance of the animal occurs.

The apparatus is a ventilated perspex box through which room air is drawn at a measured flow-rate i.e. it is open-circuit, and daily measurement of oxygen consumption may be calculated. Gas analysis is performed by a Servomex oxygen analyser, the samples submitted are selected by a time switch (Selectro) operating three mains-operated solonoid gas valves (Danfoss). The samples are as follows: (1) The exhaust gas for 52 min. (2) The exhaust gas scrubbed to remove CO_2 by carbasorb for 4 min. (3) The inlet gas for 4 min. Thus the oxygen content of the exhaust gas is monitored almost continuously and this value is compared against a room air standard every hour. The CO_2 content of the exhaust gas is calculated from its oxygen content after the removal of CO_2 (Meade & Owen-Thomas, 1973). Hence R.Q. may also be determined hourly. The output of the oxygen analyser is recorded on a chart recorder (0·5 mm/min). Activity (ultrasonic activity meter: C. F. Palmer) and food intake may be measured concomitantly.

The apparatus is particularly useful for the screening of thermogenic drugs and for comparing different strains of rats showing differences in energy metabolism.

Strain	Weight (W) (kg)	Energy expenditure (O_2 l/rat per d)	Energy expenditure (kJ/$W^{0·75}$ per d)	Energy expenditure (kJ/LBM per d)	Carcass (g fat/kg)
Zucker fat	0·545±0·034	10·3±0·4	335±22	964±70	595± 7
Zucker lean	0·266±0·004	7·2±0·2	399±12	705±19	213±13
Hooded	0·284±0·028	7·6±0·6	410±65	740±89	245±44
Sprague-Dawley	0·321±0·026	7·7±0·8	393±45	594±68	165±44
Wistar	0·274±0·013	7·8±0·8	419±47	689±69	162±19
Wags	0·210±0·005	6·4±0·6	422±42	735±85	152±14
Wild	0·180±0·010	7·7±0·6	553±33	926±59	73± 5

Data from various strains of rat (4 animals/group) ±SE of mean: the rats were adult females (300 d old) fed on oxoid diet from weaning. LBM=W−fat (kg).

M. Boroumand is a WHO fellow.

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Alterations in blood glucose concentrations in obese mice. By A. DJAZAYERY, *Department of Ecology, School of Public Health, Tehran University, Tehran, Iran*

Obesity, induced in mice by the use of a high-protein–high-fat (HP–HF) diet, gold-thioglucose (GTG) or monosodium glutamate (MSG), has been partly characterized (Miller & Parsonage, 1972; Djazayery, Miller & Stock, 1973). However, there is not much information about blood glucose changes as a result of these treatments. This study reports blood glucose measurements in mice made obese by these methods as well as in obese-hyperglycaemic (obob) mice.

Groups of weanling female mice of Ash-XP strain were made obese either by injection with GTG (0·5 mg/g body weight) or MSG (5 mg/g body weight), as reported previously (Djazayery & Miller, 1973), or by feeding a HP–HF diet (Miller & Parsonage, 1972). After 3–4 months all the groups as well as the obob group (with a similar age) were fasted over-night, killed, and their blood taken by heart puncture and analysed for glucose; also, the carcasses were analysed for fat. The results (mean values±SE) are shown below:

Treatment	Body wt (g)	*P*	Carcass fat (g/kg)	*P*	Blood glucose (mmol/l) Mean	SE	*P*
GTG	61·8	0·001	510	0·001	7·089	0·339*	0·017
MSG	36·3	n.s.	280	0·016	6·561	0·156*	0·043
HP–HF	42·3	0·004	310	0·008	7·183	0·300*	0·008
Obob	84·7	0·0001	680	0·0001	12·17	0·744**	0·00001
Control	34·7		170		5·728	0·361	

*The differences among the top three groups are non-significant.
**Highly significantly different from all the experimental groups.

All the experimental groups had significantly higher blood glucose concentrations than the controls. The amount of increase was not quite in proportion with the increase in body weight or carcass fat. Neither was it of the same magnitude: while the blood glucose level in the MSG group was only one-seventh more than that of the controls, the value for the GTG and HP–HF groups was one-third more than the control value. The very marked hyperglycaemia of the obob mice is, in fact, a characteristic of this genetic model of obesity (Mayer & Thomas, 1967).

In summary, there is hyperglycaemia of different degrees in genetic and non-genetic obesities in mice. The actual glucose increase seems to be a characteristic of the method of inducing obesity rather than a function of the increased body weight or body fat, which may suggest basic metabolic differences in the mechanism of the development of obesity in the models studied.

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Adipose tissue: contributions of nature and nurture to the obesity of an obese mutant mouse (obob). By MARGARET ASHWELL, C. J. MEADE, P. M. MEDAWAR and C. SOWTER, *Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ*

Although inherited differences of obesity have been known to exist for some time in rodents, no biochemical interpretation has yet been generally accepted (Bray & York, 1971). The aim of our investigation was to compare the contribution of the genotype of the adipose tissue and of its environment to the obesity of the mutant mouse C57 Bl/6J obob. These obese mice had been backcrossed sufficiently often onto the C57 Bl/6J background to make alloplastic transplantation possible.

Pieces of either epididymal or subcutaneous fat from lean or obese mice (about 50 d old) were transplanted to a site underneath the kidney capsule or recipient lean and obese mice. The grants were left in place for a month and then examined histologically to measure fat cell diameters from which fat cell weights were estimated (Ashwell, Priest, Bondoux, Sowter & McPherson, 1976). The results are summarized in the table:

Donor animal	*n*	Donor fat cell weight after 1 month (μg±SD)	Recipient animal	*n*	Graft fat cell weight (μg±SD)	Fat cell weight Donor *v.* Graft
Obese	3	0·504±0·207	lean	12	0·100±0·071	$P<0.001$
Obese	3	0·504±0·207	obese	7	0·480±0·151	n.s.
Lean	3	0·095±0·039	lean	11	0·102±0·048	n.s.
Lean	3	0·095±0·039	obese	6	0·577±0·437	$P<0.001$

n.s. not significant.

The fat cells of obese donor fat decrease in size in a 'lean environment' to the size typical of 'lean fat' while the cells of lean donor fat transplanted into an 'obese environment' increase to the size typical of 'obese fat'.

When both lean and obese fat tissues were transplanted onto opposite kidneys of the same mouse, the tissues acquired the cell sizes characteristic of their common host and there was no significant difference in cell size in the two grafts.

The internal environment is thus of paramount importance and inherent properties of adipose tissue are of lesser importance in determining obesity in this particular strain of mice.

A more detailed account of these results will be published in the Proceedings of the Royal Society, Series B.

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Investigations into the role of fluid in energy balance. By M. DURRANT, R. TOFT, S. MANN and J. S. GARROW, *Clinical Research Centre, Watford Road, Harrow HA1 3UJ*

Hunt, Cash & Newland (1975) have suggested that obese people who take more fluid will tend to take less food. They found that the density of intake of obese women was 4·6 kJ/ml compared with 3·0 kJ/ml in normal weight controls. They also suggested that the daily urine volume will be greater in people of low Quetelet Index (weight÷height^2) than in overweight people.

Patients were admitted to a hospital metabolic unit for 21 d for investigation. Each subject followed a similar reducing diet but had free access to acaloric fluid. A regression of average weekly urine volume against Quetelet for fifteen patients gave a slight but insignificant positive correlation (r=0·4). These findings do not support the hypothesis of Hunt *et al.*

On days 9, 10 and 14, 15 patients were confined to a direct calorimeter for 8 h daily. They were fed 1·55 MJ on days 9, 10 and 2·90 MJ on days 14, 15. This food provided densities of 4·2 kJ/g and 5·0 kJ/g respectively. On one of each of the paired days the food was diluted to 2·6 kJ/g and 3·1 kJ/g respectively by giving acaloric, flavoured drinks at mealtimes. The sequence of energy density was alternated for each pair of days, for each patient.

Patients were asked to choose the day within each pair, on which they thought the energy content was greatest. It was reasoned that patients would associate the low density day with a higher energy intake if they had experienced more satiety under those conditions.

The thirteen preferences were plotted using a sequential design (Armitage, 1960) with boundaries set at the 5% significance level. Seven of the preferences were for low density and six were for high density indicating that there was a less than 1 in 20 chance that there was a preference greater than 75% for low density. It was concluded that patients did not report a significantly increased satiety when the energy density of the food was reduced without changing the energy supplied.

During these trials, metabolic rate was measured by direct calorimetry. Paired t tests failed to show any significant difference in metabolic rate between the paired days at the different energy densities fed. These tests failed to show any metabolic effect of fluid which would affect energy balance in the patients studied.

We would like to thank Beecham Products Research Department for providing the low energy drinks, and for financial support for this investigation, and K. McPherson for help with the sequential experimental design.

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Cognitive thresholds and human body weight. By J. S. GARROW and SUSAN STALLEY, *MRC Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ*

In a previous communication (Garrow & Stalley, 1975) we reported an experimental test of the 'set point' theory for regulation of human body weight. This showed that if weight is increased by overfeeding it does not spontaneously revert to baseline level in one of us (J.G.), as the set point theory might predict. We have now continued the experiment for a duration of 1050 d to test the effect of decreasing weight below 'normal' level: the result is shown in Fig. 1. After a period of underfeeding for 31 d, with 7 kg weight loss, the subject was weighed 'blind' for another 300 d. Weight spontaneously increased to approximately the level achieved during the previous overfeeding experiment, but at this level the experiment was no longer truly blind, since the subject was now aware that he had reached an unacceptably high weight. These observations are compatible with the hypothesis that long-term regulation of body weight in some adults is achieved 'by more or less conscious effort at a stage when the change in body weight is no longer acceptable' (Garrow, 1974).

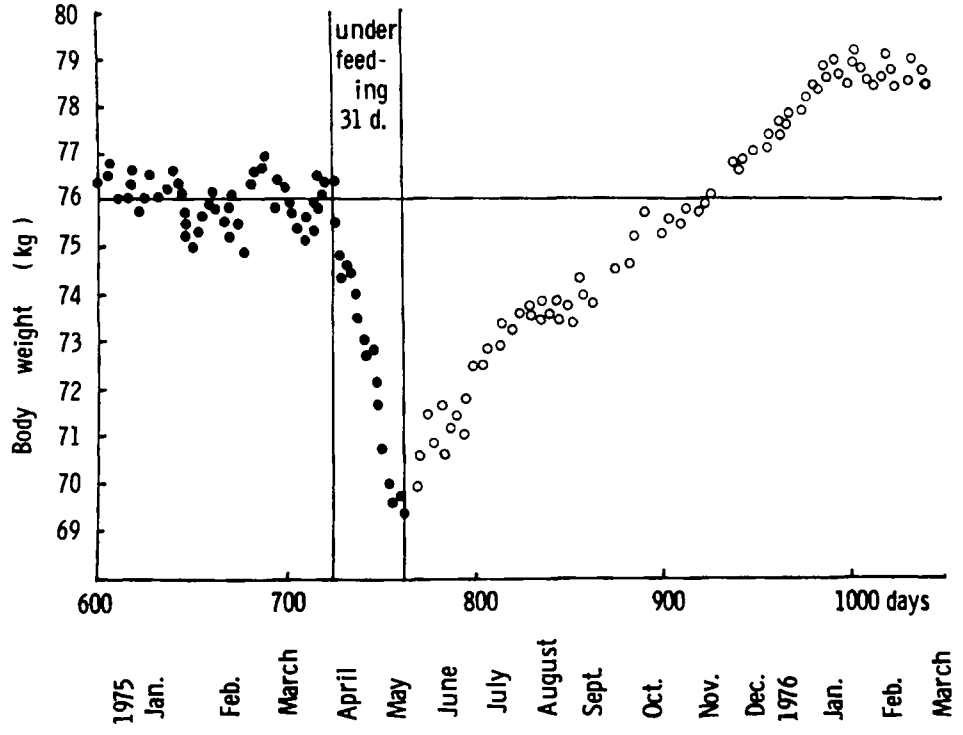

Fig. 1. Weight chart of the subject studied by Garrow & Stalley, 1975. ●, weight known to subject. ○, weight not known.

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Turnover of alanine and glycine in foetal and suckling lambs. By G. M. HATFIELD, MARJORIE K. JEACOCK, JENNY JOYCE and D. A. L. SHEPHERD, *Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ*

Tracer kinetic experiments using a single injection technique have been performed to determine the turnover of alanine and glycine in foetal and suckling lambs. Foetal aortae and posterior vena cavae were cannulated by way of the femoral vessels at about 100 d gestational age and the experiments were performed in conscious fed sheep at least 7 d after surgery. The jugular veins of the suckling lambs were cannulated and these animals were allowed free access to their mothers during the experiments. Whole-blood amino acid concentrations were determined using an enzymic method for alanine (Williamson, 1975) and a colorimetric method for glycine (Sardesai & Haydee, 1970). After separation of metabolites using thin-layer chromatography, radioactivity was measured by scintillation counting. Exponential curves were fitted to the data using a computer programme. The results were:

Amino acid	Gestational age (d)	Weight (kg)	Blood amino acid concentration‡ (μmol/l)	Turnover rate (μmol/min)	Pool mass (μmol)
Alanine	112	1·50†	296±16 (8)	47·4	284
	116	1·70†	230± 6 (10)	30·4	257
	158*	7·25	232±12 (13)	132·9	4911
Glycine	107	1·25†	463±24 (9)	49·7	664
	111	1·45†	577± 9 (13)	41·4	317
	156*	3·95	470±56 (13)	193·1	3745

*Pregnancy has been assumed to last 147 days.
†Estimated from our own unpublished data.
‡Mean values during the course of the experiment with their standard errors; number of observations in parentheses.

The turnover rate of alanine and glycine per kg body weight is similar in foetuses and lambs but is up to tenfold greater than that reported by Wolff & Bergman (1972) for the mature non-pregnant non-lactating sheep. The pool masses of alanine and glycine when related to body weight are greater in lambs than in foetuses even though the concentrations of the amino acids in whole blood are similar. That amino acid catabolism occurs in foetuses and lambs was indicated by the observation that radioactive carbon dioxide was isolated from blood in these experiments.

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Gluconeogenesis from amino acids by isolated sheep liver cells. By J. L. MORTON and P. J. BUTTERY, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD* and D. B. LINDSAY, *ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

Although gluconeogenesis from amino acids has been extensively studied in the non-ruminant, in the ruminant the published data is essentially confined to studies with the whole animal. We report some results on the production of glucose from amino acids by isolated adult sheep hepatocytes.

Cells were prepared from the caudate lobe of the liver of an adult sheep taken immediately on slaughter. The lobe was initially perfused via a vein with 150 ml of ice-cold bicarbonate buffered Weymouths medium, pH 7·4, and then with 150 ml of the same medium at 39°, oxygenated with 95% O_2–5% CO_2. Once the perfusion was established 50 mg collagenase (type II, 125–200 units/mg, Sigma Chemicals, London) was added to the perfusate. The lobe was perfused 1 h later with ice-cold medium and then sliced on a hand microtome. The slices were then incubated at 39° under 95% O_2–5% CO_2 in 500 ml bicarbonate buffered Weymouths medium at pH 7·4 containing 50 mg collagenase, for 30 min on a shaking water bath at 100 strokes/min. The suspension was filtered through gauze and then centrifuged at 100 **g** for 2 min and the pellet resuspended and washed in ice-cold bicarbonate buffered glucose-free Earles medium, pH 7·4. The cells were incubated at 39° in either glucose-free HEPES buffered Hanks medium pH 7·4, or glucose-free bicarbonate buffered Earles medium, pH 7·4. No difference between these media were noted.

Although the rates were variable between preparations [212±143 nmol glucose produced from 10 mM alanine/10^7 cells per h (mean±SEM of 5 experiments)] the ratio between glucose production for each L(−)-amino acid (10 mM), relative to that of L(−)-alanine (10 mM), was more reproducible (see table).

(Each experimental included an L-alanine incubation, taken as 100. Values are expressed as mean ±SE)

No substrate	64·9± 6·67	(5)	Leucine	84·2±3·12	(3)
Alanine	100·0	(5)	Lysine	73·3 and 82·7	
Arginine	41·3± 9·00	(4)	Methionine	76·0 and 122·2	
Asparagine	97·5±26·95	(3)	Ornithine	57·2 and 68·4	
Aspartate	136·8±16·02	(3)	Phenylalanine	68·7 and 56·5	
Citrulline	71·4 and 65·2		Proline	78·8 and 57·6	
Cysteine	78·3 and 130·5		Serine	112·3± 15·39	(4)
Glutamine	92·2±15·13	(3)	Threonine	112·8± 32·35	(5)
Glutamate	72·8±20·42	(4)	Tryptophan	71·8± 11·51	(4)
Glycine	111·1± 5·62	(3)	Tyrosine	69·9± 7·84	(3)
Histidine	102·0±10·09	(5)	Valine	83·6± 18·30	(5)
Isoleucine	76·1± 4·14	(3)	Sodium propionate	352·6±207·55	(5)

J.L.M. holds a SRC CASE studentship.

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Food intake and anthropometric measurement in pre-school children in the rain forest region of Nigeria. By D. O. NNANYELUGO and T. E. EYO, *Department of Food and Home Sciences, University of Nigeria, Nsukka, Nigeria*

The last National Nutrition Survey in Nigeria (Nutrition Survey, 1967) showed that the children living in rain forest regions showed gross growth retardation for age. In that survey, children in both urban and rural areas showed growth rates lower than 3rd percentile of Harvard standard.

In the present survey which lasted 3 months between January and March 1976, a total of 233 pre-school children from urban and rural rain forest town of Uyo were evaluated for nutritional status. The children were aged from 6 months to 5 years. Individual weighed food intakes were measured in 37 rural pre-school children out of the total number who took part in the survey. Ninety per cent of the heads of families of the children studied were subsistence farmers with a mean income of ₦400 (£320) per annum. The nutrient intakes were calculated from FAO food composition table for Africa and compared with the FAO Nutrient requirements.

Roots and tubers still provided most of the energy; protein was mainly from vegetable diets and supplemented with marginal animal protein. Energy and thiamin levels were very low, whilst, consumption of niacin and riboflavin were relatively marginal. The greatest deficiency occurred when the children were aged between 2 and 3 years. Percentages of FAO requirements are indicated in the table.

Age (yr)	Distribution	No. of subjects	Energy (MJ)	Protein (g)	Iron (mg)	Calcium (mg)	Vitamin A (iu)	Thiamin (mg)	Riboflavin (mg)	Nicotinic acid (mg)	Ascorbate (mg)
	Range		2·32–4·28	9·2–21·3	5·6–19·0	212–582	586–1600	0·28–0·52	0·38–0·86	4·10–8·20	12·6–27·6
0·5–1	Mean	12	3·36	16·0	9·46	379	978	0·42	0·63	5·95	19·1
	%		70	100	135·1	68·9	98·0	105·0	104·5	90·2	95·5
	Range		3·45–6·78	16·6–28·2	7·9–19·8	240–460	614–1124	0·24–0·54	0·42–0·84	4·0–10·0	11·0–22·4
2–3	Mean	7	4·72	23·4	13·21	329	834	0·37	0·58	7·50	18·6
	%		83	146	188·7	73·1	104·3	74·0	82·9	87·2	93·0
	Range		4·75–9·42	16·3–46·6	8·5–27·2	207–513	624–2360	0·43–1·00	0·48–1·24	4·46–14·0	16·2–56·5
4–5	Mean	18	6·80	29·3	16·08	335	1309	0·67	0·78	9·40	33·0
	%		90	144	229·7	74·4	130·9	74·4	86·7	84·7	165·0

Anthropometric measurements involving weight, height, chest, arm and head circumferences were also carried out. In all the parameters measured, the urban pre-school children showed increases which were found to be statistically highly significant.

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Assessment of nutritional status of pre-school children receiving high protein supplementation. By D. O. NNANYELUGO, *Department of Food and Home Sciences, University of Nigeria, Nsukka, Nigeria*

The first enthusiasm for high protein concentration in infant's food has faded with the realization that protein surplus to requirements will be used for energy and when the energy value of the diet is low, protein will be metabolized for energy. With the development of more protein-rich mixtures in several countries, it is now emphasized that they are to be used as supplements.

Under conditions of food shortage or where the incidence of undernutrition is high, the use of high protein supplements for pre-school children is frequently advocated, although the diet may be severely lacking in energy (WHO, 1971). Many of the high protein supplements produced are relatively low in energy content.

Investigation was currently done on pre-school children consuming high protein food designated as SW.

SW is a low income food with a pre-mix of vitamins and minerals. Its protein efficiency ratio (PER) is 2·4 and the protein content is more than 20%. The estimated energy value is 1·56 MJ (372 kcal)/100 g. SW was originally designed to satisfy the tastes of several ethnic and cultural groups in Nigeria and was primarily introduced into the country during the civil war (1967–9) as a high protein mixture for people of all ages.

A dietary and acceptability survey was carried out in three market-urban and market-rural centres to investigate the nutritional and acceptability implications of SW in the local dishes. During the survey which lasted 6 months, a total of 310 households buying SW frequently were carefully interviewed. Out of this number, 97 were selected by random sampling and a detailed individual food intake conducted on the pre-school children receiving the supplements.

The results of the survey showed that the product is fairly well accepted by several ethnic groups, but the contribution of SW to the total daily energy intake is small compared to that supplied by other nutrients.

Group	Ages (yr)	Mean weight (kg)	Amount of SW consumed (g)	Energy intake (MJ/d)	Energy intake (kJ/kg body wt per d)	Protein intake (g)	Protein intake (g/kg body wt per d)	P:E	Fat (g/d)	Carbohydrate (g/d)
Without SW	0·5–1	6·8	—	4·18	614	16·0	2·35	6·4	18·0	172
	2–3	10·4	—	4·82	463	23·4	2·13	8·2	18·7	270
	4–5	13·6	—	7·90	581	29·3	2·11	6·2	24·0	264
Plus SW	0·5–1	6·8	26	4·68	615	21·9	3·22	7·8	19·9	190
	2–3	10·9	30	5·22	479	29·0	2·64	9·3	20·5	291
	4–5	13·9	72	9·00	647	43·7	3·14	8·2	28·0	307

P:E, (protein energy ÷ total energy) × 100.

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Retinol-binding protein and pre-albumin in serum of children with xerophthalmia before and after treatment. By A. PIRIE, M. COBBY and G. VENKATASWAMY, *Nuffield Laboratory of Ophthalmology, Oxford and Nutrition Rehabilitation Centre, Government Erskine Hospital, Madurai, India and* J. GLOVER and S. LARGE, *Department of Biochemistry, Liverpool University, Liverpool*

We have studied retinol-binding protein (RBP) in serum of children who show clinical signs of xerophthalmia due to vitamin A deficiency. Estimations of total RBP were made by the method of single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) and of holo-RBP by the method of Glover, Moxley, Muhilal & Weston (1974). Most of the children were severely malnourished and less than 60% of their expected wt/age.

We confirmed the results of Smith, Goodman, De Witt, Arroyave & Viteri (1973) that malnourished children have low levels of RBP and of prealbumin (PA) in their serum. Those children with severe corneal xerophthalmia had significantly less RBP than those with milder conjunctival xerophthalmia. The lowest values found were in three children who had recently had either measles, mumps or whooping cough.

An injection of 100 000 I.U. of water-miscible retinol-palmitate did not raise the blood RBP to a normal level within 24 h. The children were fed a vegetarian diet providing about 4 g protein/kg and 4–6 mg carotene/d. After about a week on this diet with or without further vitamin A, their blood RBP reached a normal level.

Holo RBP was estimated in some blood samples taken at various time intervals from the same children and, in general, changed in parallel with total RBP.

The results suggest that lack of protein from which to synthesise RBP may, as previously suggested by Smith, Goodman, De Witt, Zaklama, Gabr, El Maraghy & Patwardhan (1973) in malnourished children, be an important factor in the failure to transport retinol in xerophthalmic children. The results of the dietary treatment on corneal and conjunctival xerophthalmia and on weight gain will be described.

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Determination of vitamin D in butter and ghee by chick bioassay. By PENELOPE GORDON, K. SHARMA and T. G. TAYLOR, *Department of Physiology and Biochemistry, Faculty of Medicine, The University, Southampton SO9 3TU*

Ghee is an important source of vitamin D in the diet of Asian immigrants in Britain, in whom rickets and osteomalacia are a serious cause for concern. Although cholecalciferol is known to be fairly stable to heat, its precise stability during the conversion of butter to ghee has not been determined experimentally.

The standard diets for the chick bioassay contained (g/kg): maize (350), soya-bean meal (371), dried yeast (50), $CaCO_3$ (13), NaH_2PO_4 (9), NaCl (5), methionine (1), ZnO (0·12), $MnSO_4$ (0·16), vitamin-free margarine (200) plus 600 μg retinol/kg. Three standard diets containing 1·25, 2·5 and 5·0 μg/cholecalciferol per kg were included in each assay. In the experimental diets the margarine was replaced by butter or by the ghee prepared from 200 g butter. The ghee was made in a saucepan over a burner in the traditional manner.

The chicks were housed in a four-tier electrically heated battery brooder and each tier was divided into four compartments. Two brooders were used, one for the butter and one for the ghee diets and the experimental design was in the form of a 4×4 Latin Square, with one experimental and three standard diets each replicated four times.

For the first 7 d after hatching all the chicks were fed on a diet containing 1 μg/cholecalciferol per kg and those weighing between 85–95 g were selected and allocated at random to the experimental treatments. There were ten chicks per compartment and the experimental diets were given for 21 d. The chicks were weighed weekly in groups.

The final mean weights of the butter- and ghee-fed chicks were 200 g and 204 g respectively and this difference was non-significant. The regression equation relating chick weight to log dose of cholecalciferol was $y=112+56·6\ x$, where y=chick weight (g) at 28 d of age, x=log cholecalciferol (iu/kg diet). Taking the mean chick weight of 202 g this equation gives a value of 0·975 μg/cholecalciferol per kg diet (4·88 μg/kg butter).

In a pilot experiment the butter was shown to contain almost 25 μg/cholecalciferol per kg, confirming the variability in the vitamin D content of butter.

In a previous experiment with over 300 chicks, which was similar in design to the one described above, the final mean weights of the butter- and ghee-fed chicks were 184 g and 193 g, respectively, and we conclude that when butter is made into ghee the cholecalciferol is quite stable.

Sterols in British shellfish. By ANN SCHULZE and A. S. TRUSWELL, *Nutrition Department, Queen Elizabeth College, Campden Hill, London W8 7AH*

A current corner of confusion in therapeutic dietetics is the status of shellfish in diets designed to lower plasma cholesterol. Many diet sheets forbid all shellfish though some modern ones permit shellfish in moderation except shrimps (Fredrickson, Levy, Bonnell & Ernst, 1975). There is insufficient analytical data on the sterols of edible shellfish in general (Feeley, Criner & Watt, 1972) and of British shellfish in particular.

Shellfish were purchased fresh at Billingsgate market in June 1976 or bought frozen if fresh were not obtainable. Lipids were extracted from freeze-dried samples with chloroform–methanol (2:1, v/v). 'Cholesterol' was measured by the method of Abell, Levy, Brodie & Kendall (1952). The unsaponifiable fraction was analysed by gas–liquid chromatography (GLC) on 3% OV-17 at 260°.

'Cholesterol' (i.e. total sterol) contents were (mg/100 g cooked except oysters): (in Gastropoda) periwinkles 139, whelks 105; (in Lamellibranchia) cockles 107, oysters (flat) 90, scallop, fresh 82, frozen 160; (in Cephalopoda) squid 211; (in Crustacea) crab abdomen 'paste' 167, crab claw meat 69, lobster 129, prawn (frozen) 178, scampi (frozen) 107, shrimp 198. For comparison, cod contained 41 and sardines 67 mg cholesterol/100 g.

On GLC the cholesterol peak made up 90% or more of total sterols, except in frozen scallop it was only 32%, in fresh scallop 39%, cockles 37%, oysters 52% and periwinkles 72%. In these seafoods therefore true cholesterol was only 51, 32, 40, 47 and 101 mg/100 g respectively. The other sterols were differing mixtures of 24-methylene cholesterol, brassicasterol, 22-dehydrocholesterol, desmosterol, etc. Some of the minor peaks could not be identified. The biological effect of most of these sterols is as yet unknown, although desmosterol is atherogenic, but scallop meal does not raise plasma cholesterol in chicks (Idler & Wiseman, 1972).

Other dietetic considerations are that the glyceride fatty acids of shellfish may include moderate to high proportions of polyunsaturated fatty acids, that most people only eat shellfish on special occasions and that shrimps are usually eaten potted with butter.

Dr L. J. Goad (Biochemistry Department, Liverpool University) kindly supplied several rare standards.

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Niacin in instant coffee. By P. OKUNGBOWA, M. C. F. MA and A. S. TRUSWELL, *Nutrition Department, Queen Elizabeth College, Campden Hill, London W8 7AH*

It is not generally realized that coffee is a useful source of niacin, which is formed from trigonelline when the beans are roasted (Bressani & Navarrete, 1959), and that this is biologically available for man (Goldsmith, Miller, Unglaub & Kercheval, 1959). However, we could not find any figures for the niacin content of instant coffee powders in Britain, the usual material used for making the beverage nowadays.

Before our analysis of instant coffees we examined the thin-layer chromatographic methods for niacin that have been reported in the last 15 years. We found that 4-chloro-1,3-dinitrobenzene followed by sodium hydroxide (Washüttl, 1970), or cyanogen bromide followed by *p*-aminobenzoic acid (Kodicek & Reddi, 1951) are both efficient reagents for detection and quantifying nicotinic acid and nicotinamide, but the former is preferable where the fume cupboard has to be shared because it is less toxic. Other reagents were less effective. Of three methods for decolorizing coffee that we tried, the magnesium oxide method (Kogan, DiCarlo & Maynard, 1953) was best. Following this, propan-1-ol–10% ammonia (95:5, v/v) was a good solvent system. Spots were quantified by measuring their area; each unknown was run in duplicate with 4 standards (1, 2, 3 and 5 μg) on a plate; nicotinamide was quantified separately from nicotinic acid and small losses during decolorization were corrected for.

We found that six instant coffee brands contained mean 24 mg nicotinic acid and 6 mg nicotinamide/100 g powder and decaffeinated coffee a similar amount. Hence the average cup of coffee contains approximately 0·6 mg niacin and 5 cups per day provide one-fifth of the recommended intake, all in available form. The National Food Survey underestimates this contribution.

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Dietary induced renal damage in the rat. By S. S. KANG, R. G. PRICE, K. R. BRUCKDORFER, N. A. WORCESTER and J. YUDKIN, *Queen Elizabeth College, University of London W8; Department of Biochemistry and Chemistry, Royal Free Hospital Medical School, London WC1; and Servier Research Institute, Horsenden Lane South, Greenford, Middlesex*

Cohen & Rosenmann (1971) demonstrated that long-term feeding of a sucrose-rich diet resulted in diffuse intercapillary glomerulosclerosis. Previous studies by our group revealed that a constant feature of sucrose feeding was a significant enlargement of the kidney (Kang, 1973). We report on a study designed to determine whether diets with sucrose, together with either butter or poly-unsaturated margarine cause possible renal damage in rats as indicated by an increase in the urinary excretion of the enzyme *N*-acetyl-β-glucosaminidase (NAG) (*EC* 3.2.1.30) (Dance, Price, Cattell, Lansdell & Richards, 1969). The diets consisted of (g/kg) starch or sucrose 550, butter or margarine 200, casein 160, salt mixture 40, cellulose 30, vitamin mix 20.

Sixty-four male Sprague-Dawley rats were randomly divided into 4 groups of 16, and each group fed *ad lib.* one of the diets for 8 months. One day before the end of the experiment, urine was collected for 8 h from 8 rats of each group, its volume measured, and enzyme activity assayed. The rats were killed by cervical dislocation, and the kidneys removed, decapsulated, weighed and fixed in formalin for subsequent examination by light and electron microscopy.

Table 1. *Effect in rats of different dietary fats and dietary carbohydrates on urinary N-acetyl-β-glucosaminidase (NAG)*

Diet		Kidney wt		Urinary NAG	
Carbohydrate	Fat	(g)	SEM	(units/8 h)	SEM
Starch	butter	3·15	0·1	178	38
Sucrose	butter	3·70	0·11	281	23
Starch	margarine	3·15	0·11	202	32
Sucrose	margarine	3·73	0·14	268	45

Significant differences (by analysis of variance), Kidney weight: Starch *v.* sucrose $P<0.005$; NAG activity: Starch *v.* sucrose $P<0.025$.

No significant differences between the groups were found in body weight or in urinary volume. Compared with rats fed starch, those fed sucrose had heavier kidneys, and excreted more enzyme in the urine. The difference in dietary fat made no difference to kidney weight or enzyme excretion.

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