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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Twenty-eighth Meeting of the Nutrition Society was held at the University of Surrey, Guildford, on Thursday, 19 *April* 1979, *when the following papers were read:*

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Bone demineralization in rats caused by polyunsaturated margarine. By K. R. BRUCKDORFER and NANCY A. WORCESTER, *Department of Chemistry and Biochemistry, Royal Free Hospital School of Medicine, London WC2* and J. YUDKIN, *Department of Physiology, Queen Elizabeth College, London W8*

The feeding of rats with diets containing either starch or sucrose as the carbohydrate, and either butter or polyunsaturated ('soft') margarine as fat, showed that, compared with starch, sucrose produced much more renal calcinosis and a higher urinary excretion of phosphate, but that the nature of the dietary fat produced no difference in these characteristics (Kang *et al.* 1979). However, we now report that the mineral composition of the bones is affected by the nature of the dietary fat but not by the nature of the carbohydrate.

Four groups, each of ten Sprague–Dawley male rats weighing approximately 100 g, were given purified diets containing (g/kg) 550 either corn starch or sucrose, 200 either butter or soft ('Flora') margarine, 160 casein, and additions of mineral mix, vitamins and cellulose (Kang *et al.* 1979). After 8 months the rats were killed, and the right femurs removed, cleaned, extracted with ether, ashed and assayed for calcium and magnesium. Since the composition of the bones was not affected by the nature of the dietary carbohydrate, we have combined the results of the groups given starch or sucrose (see Table).

Mineral content (mg/g) of defatted femurs of rats fed on diets containing butter or polyunsaturated margarine for 8 *months*

(Mean values with their standard errors for twenty rats/group)

	Butter-fed rats		Margarine-fed rats		
Mineral	Mean	SEM	Mean	SEM	*P*
Ca	282	13	212	14	<0·001
Mg	4·4	0·2	3·8	0·2	<0·05

The mineral content of the femurs of the butter-fed rats was similar to that of rats fed the laboratory stock diet, so that it seems reasonable to conclude that polyunsaturated margarine leads to some degree of demineralization of bone in the growing rat. Compared with butter, polyunsaturated margarine has a higher content of vitamins A and D and of polyunsaturated fatty acids. Whether the demineralization is caused by any of these factors, or some other factor, remains to be determined.

Kang, S. S., Price, R. G., Yudkin, J., Worcester, N. A. & Bruckdorfer, K. R. (1979). *Br. J. Nutr.* **41**, 65.

0029–5651/79/3830–328A $00.35

Site differences in fat cell size–a transplantation study. By C. J. MEADE and MARGARET ASHWELL, *Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey and Clinical Research Centre, Watford Road, Harrow*

Many people are more concerned about the distribution of excess fat than its total amount. The New Zealand obese mouse provides a model for unequal distribution of excess fat. In middle life, the gonadal fat pads are enlarged, chiefly as a result of increased fat cell size, whilst subcutaneous cells are much smaller.

We have transplanted subcutaneous or gonadal fat to a third, common site in the same mouse, in order to see if it was intrinsic differences or location of fat that caused the difference in fat cell size. Fat was transplanted under each kidney capsule by a technique described earlier (Ashwell *et al.* 1977). Grafts were removed after 1, 2 or 3 months and fixed sections used for measurement of maximum cell diameter, from which cell mass could be calculated.

Changes in mass of gonadal or subcutaneous fat cells following transplantation beneath the kidney capsule

(Mean values with their standard deviations; no. of animals in parentheses)

Period transplant in place (months)	Sex of host mice	Fat cell masses (μg)								Mass of fat cells at gonadal site / Mass of fat cells at subcutaneous site	
		Before transplant				After transplant					
		gonadal		subcutaneous		gonadal		subcutaneous			
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Before transplant	After transplant
1	♂ (6)	0·266	0·105	0·186	0·081	0·164	0·108	0·116	0·053	1·86	1·44
2	♂ (19)	0·223	0·105	0·125	0·081	0·181	0·047	0·166	0·052	1·82	1·17
3	♂ (16)	0·246	0·105	0·109	0·081	0·215	0·058	0·232	0·043	2·00	0·96
1	♀ (5)	0·242	0·077	0·075	0·031	0·069	0·019	0·073	0·038	3·13	1·13
3	♀ (5)	0·258	0·077	0·093	0·031	0·232	0·046	0·193	0·040	2·88	1·23

As the Table shows, the marked and statistically significant differences in sizes between fat cells from gonadal and subcutaneous sites almost, but not quite entirely, disappeared upon transplantation to a common site. After 1 month in the host this was largely because gonadal cells had shrunk in the new site, but after grafts were 2 or 3 months in place there was also significant enlargement of cells of subcutaneous origin.

Thus it is largely differences in location rather than in the fat tissue itself which account for the differences in size between subcutaneous and gonadal cells.

Although hormone concentrations are likely to be identical in blood bathing either site, total amount of hormone available will also depend upon blood flow. Differences in blood supply are, with differences in nervous supply, possible candidates for 'local' factors influencing cell size.

Ashwell, M., Meade, C. J., Medawar, P. B. & Sowter, C. (1977). *Proc. R. Soc.* B. **195**, 343.

0029·6651/79/3830–328A $00.35

The use of ruthenium (II) phenanthroline and chromium-EDTA as markers in digestibility studies with rabbits. By KUNJLATA AMIN and A. G. STEPHENS, *Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG6 2AJ*

Before using ruthenium (II) phenanthroline (Ru-P) and chromium-EDTA (Cr-EDTA) as markers in a study of digestion in rabbits the effect of adding the non-radioactive forms of these markers on digestibility has been studied.

Groups of four New Zealand White rabbits, live weight 2·5–3·0 kg, were maintained on one of two commercial pelleted laboratory rations, (Diet 18, E. Dixon and Sons Ltd, Ware and RAG(M), Labsure Animal Foods, Dorset). Food intake was controlled to be 100 g/d. Water was available at all times. A preliminary period of 5 d was allowed and then faeces were collected for 7 d. This procedure was repeated with the incorporation of Ru-P (14 mg Ru/kg DM food) and Cr-EDTA (350 mg Cr/kg DM food). Dry matter contents of food and faeces were determined by freeze drying to a constant weight. Ruthenium was estimated by X-ray fluorescence spectrometry (Evans *et al.* 1977).

The effect of incorporating Ru-P and Cr-EDTA on the digestibility of dry matter in rations for rabbits

(Mean values with their standard errors for four replicates)

		Ration			
		Diet 18		RAG(M)	
	Method of calculation	Mean	SE	Mean	SE
Without any markers	Total collection	0·629	0·008	0·629	0·006
With Ru-P and Cr-EDTA	Total collection	0·728	0·002	0·635	0·001
With Ru-P and Cr-EDTA	Indicator	0·734	0·021	0·628	0·021

The Table shows the apparent digestibility coefficients for the dry matter determined with or without incorporation of the markers. Calculation of digestibility by total collection method or by reference to marker concentration is also presented. There was a significant increase ($P<0·01$) in the digestibility of dry matter for diet 18 when the markers were added, but no difference was observed for RAG(M). No differences between the two methods for calculating digestibility were observed.

At the levels of incorporation of the markers used, it is concluded that no adverse effects on the digestibility of dry matter results. The unexpected increase in digestibility of diet 18 can be explained by an initial reduction in food intake when the markers were added.

Evans, C. C., MacRae, J. C. & Wilson, S. (1977). *J. agric. Sci. Camb.* **89**, 17.

0029–6651/79/3830–328A $00.35

A simple and cheap respiration chamber for long-term studies of energy expenditure in human beings. By M. I. GURR, *National Institute for Research in Dairying, Shinfield, Reading, RG2 9AT* and M. P. ROBINSON and D. MALTBY, *Nutrition and Agriculture Division, Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ*

In connexion with a programme of work on the relative importance of energy expenditure and intake on the long-term regulation of energy balance in man, we constructed a respiration chamber to be described here. The chamber, measuring $3{\cdot}6\times2{\cdot}5\times2{\cdot}2$ m was constructed within 2 d, of tubular steel frame covered with thick polyethylene that was either welded or taped at the joins. A domestic vacuum cleaner provided the air flow, the rate of which was measured by a standard gas meter. Oxygen and carbon dioxide in the incoming and outgoing gases were measured by paramagnetic and infra-red gas analysers respectively. In a series of twelve experiments the recovery of carbon dioxide from the chambers was $87{\cdot}1\pm2{\cdot}7\%$. The chamber was furnished with a desk, chair and bed, leaving adequate room for exercising. In two experiments, each lasting 5 h, the heat production of a young female human subject (following exactly the same feeding and exercise routine on each occasion) was calculated from the oxygen consumed and carbon dioxide produced, using the equation (Brouwer, 1965):

Heat production (kJ) $= 16{\cdot}18\times O_2+5{\cdot}02\times CO_2-2{\cdot}17\times CH_4-5{\cdot}99\times$ urinary N

Results were as follows:

Experiment	Flow rate (l/h)*	Heat produced (MJ/h)	O_2 consumed (l/h)	CO_2 produced (l/h)
1	7117	0·384	18·99	16·86
2	7169	0·390	19·46	16·50

*Corrected to NTP and corrected for water vapour pressure.

The overall cost of the chamber was about £200 but this did not include the cost of gas analysers which were part of an animal calorimetry installation. The chamber appears to give reproducible results within our limited experience and has the advantage of cheapness, roominess, ease of construction and portability. It seems well suited to long-term studies on a limited budget.

The authors are grateful to Drs M. Cawthorne and D. S. Miller for showing us and discussing with us a similar instrument that they have been constructing.

Brouwer, E. (1965). *Energy metabolism* p. 441, [K. L. Blaxter, editor]. London: Academic Press.

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Site of absorption of magnesium from the ovine digestive tract. By T. J. FITT, K. HUTTON and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Studies (Pfeffer *et al.* 1970; Bertoni *et al.* 1976; Horn & Smith, 1978) have provided evidence that the major site of absorption of magnesium occurs before the small intestine in the ruminant.

A semi-purified diet was formulated which, when fed at 792 g dry matter/d in two equal portions supplied only 0·084 g/d Mg. Additional Mg, as $MgCl_2.6H_2O$ (0·44g Mg) was either incorporated daily in the feed or infused into a specific site of the digestive tract in 500 ml water. Each experiment conformed to the following pattern; 5 d Mg in feed or infused into the rumen followed by 6 d in which the Mg was infused into a particular site of the digestive tract and finally 3 d in which the Mg was given in the feed or infused into the rumen. Blood samples were withdrawn daily and plasma Mg determined. An outline of the experiments carried out and plasma Mg levels observed on the last day of each sub-period are given below:

(Mean values with their standard errors; site of Mg administration in parentheses)

		Period of experiment					
		Days 1–5		Days 6–11		Days 12–14	
Experiment	No. of animals	Mean	SE	Mean	SE	Mean	SE
1	6	(rumen) 20·4	0·53	(proximal duodenum) 11·2	0·86	(rumen) 20·0	0·22
3	3	(rumen) 21·6	1·42	(terminal ileum) 11·4	0·59	(rumen) 20·3	0·50
5	4	(food) 19·0	0·16	(abomasum) 10·5	0·40	(food) 18·3	0·45
7	3	(food) 18·8	0·52	(omasum) 17·8	0·11	(food) 18·8	0·20

During days 1–5, when the Mg was given via the rumen, (or in the food) plasma Mg levels were maintained. Subsequently administration of the Mg via the abomasum, proximal duodenum or terminal ileum resulted in steady falls in plasma Mg to levels indicative of the hypomagnesaemic state. Return of the site of Mg administration to the rumen or in the feed restored plasma Mg levels. When the Mg was infused into the omasum during days 6–11 no such fall in plasma Mg was observed. Similar results were obtained when a higher level of Mg (1·42 g/d) was given.

It would appear that the major site of Mg absorption is the omasum and possibly reticulo-rumen.

Bertoni, G., Watson, M. J., Savage, G. P. & Armstrong, D. G. (1976). *Zoot. Nutr. Anim.* **2**, 107.
Horn, J. P. & Smith, R. H. (1978). *Br. J. Nutr.* **40**, 473.
Pfeffer, E., Thompson, A. & Armstrong, D. G. (1970). *Br. J. Nutr.* **24**, 197.

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The effect of increasing potassium intake on absorption of magnesium by sheep. By R. C. MACGREGOR and D. G. ARMSTRONG, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Four sheep equipped with rumen cannulas and re-entrant cannulas into the proximal duodenum and terminal ileum were given each of four semi-purified diets containing increasing levels of potassium but of otherwise constant composition, including magnesium as $MgCl_2.6H_2O$, in a Latin Square design at 1 kg feed (air-dry basis)/d. Net amounts of Mg and other elements entering and leaving the small intestine, excreted in the faeces and in the urine daily were measured. In addition, the amount of Mg bound to the microbial fraction entering the small intestine was determined. Some results are given below:

	Diet			
	1	2	3	4
K intake (g/d)	6·23	13·56	21·23	38·70
Mg intake (g/d)	1·25	1·24	1·23	1·24
Apparent availability of Mg	0·212 [a]	0·226 [a]	0·238 [a]	0·157 [b]
Net absorption prior to small intestine (g/d)				
Mg	0·44 [a]	0·42 [a]	0·35 [ab]	0·20 [b]
K	−1·29 [a]	5·05 [b]	9·11 [c]	18·94 [d]
Na	−6·22 [a]	−4·74 [b]	−4·63 [b]	−4·15 [b]
Non-microbial Mg entering small intestine (g/d)	0·21 [a]	0·33 [ab]	0·31 [ab]	0·40 [b]
K:Na in microbial-free rumen liquor	0·87 [a]	1·00 [a]	1·89 [a]	5·49 [c]
Net retention of Na (g/d)	1·26 [a]	1·03 [b]	0·50 [c]	0·16 [d]

a, b, c, d. Values with unlike superscripts differed significantly ($P<0·05$).

Apparent availability of Mg $(\frac{\text{feed-faeces}}{\text{feed}})$ was depressed at the highest level of K fed in agreement with the findings of Newton *et al.* (1972). Net absorption of Mg prior to the small intestine declined at the higher levels of K fed and this decline was associated with a markedly enhanced uptake of K prior to the small intestine, and was reflected in an increased amount of non-microbial Mg entering the small intestine. The K:Na (molar basis) in microbial-free rumen fluid supernatant, increased steadily from diets 1 to 4, while retention of Na fell steadily.

These results show that when dietary intake of K is increased, with constant intake of Na, Mg absorption, which occurs in the reticulo-rumen and/or omasum (Fitt *et al.* 1979) is depressed. The effect appears to be associated with a marked change in K:Na in the rumen fluid, and enhanced K absorption prior to the small intestine.

Fitt, T. J., Hutton, K. & Armstrong, D. G. (1979). *Proc. Nutr. Soc.* **38**, 65A.
Newton, G. L., Fontenot, J. P., Tucker, R. E. & Polan, C. E. (1972). *J Anim. Sci.* **35**, 440.

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Fatty liver and infertility in dairy cows in early lactation. By I. M. REID, C. J. ROBERTS, R. A. COLLINS and SALLY M. DEW, *Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire RG16 0NN*

Mobilization of fat depots of dairy cows in early lactation resulted in accumulation of fat in liver and muscle cells (Roberts *et al.* 1979). However, the extent of fat accumulation in liver cells varied considerably. Of the twenty cows described previously (Roberts *et al.* 1979), ten had <20% fat in the liver parenchyma at 1 week after calving (mean $12{\cdot}5 \pm 1{\cdot}5$%) and were classified as having a mild fatty liver. The remaining ten cows all had >30% fat in the liver parenchyma (mean $46{\cdot}0 \pm 3{\cdot}2$%) and were classified as having a severe fatty liver. There was no difference in age or previous milk yields between the two groups.

The cows in the severe fatty liver group mobilized significantly more adipose tissue during the 8 weeks after calving as indicated by increased plasma non-esterified fatty acid (NEFA) concentrations and increased loss of fat depth when compared with the cows with mild fatty livers (see Table). Blood glucose concentrations were significantly lower in the cows with severe fatty liver. Therefore, the fatty liver is part of a fat mobilization syndrome in response to the energy deficit of early lactation.

Mean blood concentrations of metabolites, change in fat depth and fertility measurements in cows with mild or severe fatty liver

(Mean values with their standard errors)

	Mild <20% fat		Severe >30% fat	
	Mean	SEM	Mean	SEM
Blood glucose (mmol/l)†	2·38	0·06	2·14	0·08*
Plasma NEFA (μ equiv/l)†	117·3	7·96	196·4	3·93***
Change in fat depth (mm)‡	−3·10	1·26	−8·22	1·53*
Calving interval (d)§	359·0	8·1	395·5	11·3*
Services to conception§	1·73	0·18	2·39	0·18*

*Significantly different from mild group $P<0{\cdot}05$.
***Significantly different from mild group $P<0{\cdot}001$.
†Mean of weeks 1–8 after calving.
‡Change in fat depth between 1 and 8 weeks after calving.
§Mean of all previous lactations.

An interesting finding was a relationship between fatty liver and infertility. The cows with severe fatty livers had a history of infertility in previous lactations as shown by a significantly ($P<0{\cdot}05$) longer mean calving interval and significantly greater number of services to conception when compared to the cows with mild fatty liver. We are attempting to confirm these preliminary findings of a link between liver function and infertility in a larger number of cows and to investigate other consequences of excessive fat mobilization in early lactation.

Roberts, C. J., Reid, I. M., Pike, B. V. & Turfrey, B. R. (1979). *Proc. Nutr. Soc.* **38**, 68A.

0029–6651/79/3830–328A $00.35

Tissue mobilization in dairy cows in early lactation. By C. J. ROBERTS, I. M. REID, BRENDA V. PIKE and B. R. TURFREY, *Agricultural Research Council, Institute for Research on Animal Diseases, Compton, Newbury, Berkshire RG16 oNN*

High-yielding dairy cows undergo a period of energy deficit in early lactation and mobilize reserves in body tissues for milk production. We are studying the effects of this mobilization on liver, muscle and adipose tissue of cows in one of this Institute's autumn calving herds.

Twenty Friesian cows in their 3rd, 4th and 5th lactation, and giving at least 5,500 kg of milk in the previous lactation, were studied for 8 weeks before and 8 weeks after calving. Blood samples were taken weekly and samples of liver, trapezius muscle and subcutaneous adipose tissue were obtained by biopsy at 1 and 8 weeks after calving.

The average extent of fat mobilization in the first 8 weeks of lactation can be seen in the Table. The depth of the subcutaneous adipose tissue layer behind the shoulder was reduced by half and there was a clear reduction in the size of measurable adipocytes. Plasma non-esterified fatty acid (NEFA) concentrations rose steeply at calving and returned to pre-calving concentrations by the seventh week after calving.

Changes in blood and tissue measurements before and after calving

(Mean values with their standard errors)

	Pre-calving value		1 week post-calving value		8 week post-calving value	
	Mean	SEM	Mean	SEM	Mean	SEM
Fat depth (mm)			13·4	1·21	7·8	1·22***
Adipocyte diameter (μm)			118·6	2·20	105·4	3·58***
Plasma NEFA (μ equiv/l)	109·2	14·03***	239·3	24·51	89·6	7·78***
Blood glucose (mmol/l)	2·29	0·045*	2·09	0·081	2·57	0·067***
Liver fat (%)			29·9	4·63	8·0	2·76***
Muscle fibre area (μm^2)			3103	104·3	2494	113·0***

*Significantly different from 1 week post-calving value, $P<0·05$.
***Significantly different from 1 week post-calving value, $P<0·001$.

Fat mobilization resulted in fat deposition in liver and muscle, as found in the biopsy samples taken 1 week after calving. In liver 29·9% of the liver cell volume was occupied by fat, and in muscle numerous discrete fat droplets were present throughout the sarcoplasm of the Slow Oxidative type of fibres. There was a reduction in muscle fibre size of about 20% between 1 and 8 weeks after calving.

There were considerable variations between cows in the extent and consequences of tissue mobilization. These variations will be discussed in a separate paper (Reid *et al.* 1979).

Reid, I. M., Roberts, C. J., Collins, R. A. & Dew, S. M. (1979). *Proc. Nutr. Soc.* **38**, 67A.

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The use of dye binding for the concurrent determination of protein and reactive (available) lysine in leaf protein concentrate. By ANN F. WALKER, *Department of Food Science, University of Reading, Reading RG1 5AQ*

Work is being carried out at the University of Reading on the use of leaf protein concentrate (LPC) for human and animal diets. These studies include the effects of juice holding time, precipitation methods and drying techniques on the nutritional value of the protein. The availability of lysine can be used to assess processing damage (Carpenter, 1974) and recently dye-binding methodology has been used for this purpose. The Σ–NH_2 group of lysine can be blocked by various reagents (Jones, 1974), including propionic anhydride in the presence of sodium acetate (Hurrell & Carpenter, 1976). Reactive lysine is determined by the difference in dye bound before (dye-binding capacity or DBC) and after blocking the Σ–NH_2 group (the dye-binding difference or DBD-lysine).

The Foss FHI-1 dye-buffer reagent (Foss Electric (UK) Ltd, The Chantry, Bishopthorpe, York), containing C.I. Acid Orange 12 in an oxalic acid–acetic acid–phosphate buffer was used with conventional spectrophotometry and laboratory apparatus. Reactive lysine was determined for two groups of LPC samples. Group 1 comprised twenty samples produced to minimize nutrient loss and Group 2 were twenty-eight samples produced by thermal-drying techniques of varying severity.

Four samples from Group 1 were used to establish the experimental conditions. Investigations included the effect of dye uptake on time of shaking the dye with the sample, varying sample weight and sodium acetate and propionic anhydride addition.

The dye-bound protein (from DBC values) gave a value of 0·97 when compared with tungstic-acid precipitated nitrogen (×6·25) for Group 1 samples. For Group 2 samples, where thermal processing was severe, DBC values were reduced as well as DBD-values. A comparison of DBD-lysine with fluorodinitrobenzene (FDNB)-reactive lysine by the Booth (1971) modification of the Carpenter (1960) method, gave a r value of 0·90, which was a better estimate of reactive lysine (when compared to FDNB-reactive lysine) than the DBC value (r 0·83).

Similar studies on cowpea seeds suggest that the method has more general application to food materials. It is most important that the conditions of dye uptake be established fully before the method is applied to a particular foodstuff.

Grateful acknowledgement is made to the Wolfson Foundation for financial assistance.

Booth, V. H. (1971). *J. Sci. Fd Agric.* **22**, 658.
Carpenter, K. J. (1960). *Biochem. J.* **77**, 604.
Carpenter, K. J. (1974). In *Nutrients in processed foods—proteins*, p. 99 [P. L. White and D. C. Fletcher, editors]. American Medical Association. Acton, Massachusetts: Publishing Sciences Group Inc.
Hurrell, R. F. & Carpenter, K. J. (1976). *Proc. Nutr. Soc.* **35**, 23A.
Jones, G. P. (1974). PhD Thesis, University of Reading.

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The effect of guar gum on blood alcohol levels following gin and tonic consumption. By JACKI TREDGER[1], J. WRIGHT[2*] and V. MARKS[1,2], *Department of Biochemistry, University of Surrey, Guildford, Surrey*[1], *Departments of Clinical Biochemistry and Medicine, St. Luke's Hospital, Guildford*[2]

When taken in conjunction with a meal, guar gum flattens the post-prandial blood glucose curve in both normal and diabetic subjects and is thought to reduce the rate of absorption of products of digestion by virtue of its viscosity (Jenkins *et al.* 1977).

As part of our investigations into the effects of alcohol consumption, six subjects consumed within 1 h the equivalent of three double measures of gin and tonic and 8 cheese scones, on one occasion with, and on the other without, the addition of 14·5 g guar gum. The ingestion of guar gum reduced the insulinaemia and produced a flatter mean post-prandial blood glucose curve (see Table). Reactive hypoglycaemia sometimes follows the ingestion of alcohol and rapidly assimilable carbohydrate (O'Keefe & Marks, 1977). In two subjects ingestion of guar gum abolished the clinical and biochemical indications of the reactive hypoglycaemia they experienced in its absence. The mean blood alcohol levels tended to be higher in the presence of guar gum and caused the two subjects referred to above to exhibit behaviour indicative of a more severe degree of intoxication. These results suggest that the effects of guar gum may not be solely due to its viscosity.

Mean blood glucose, alcohol and plasma insulin levels after consuming 50 *g alcohol in* 110 *ml tonic water and a snack with or without the addition of* 14.5 *g of guar gum*

Time (min)	Glucose (mmol/l)		Insulin (mU/l)		Alcohol (mg/dl)	
	C	T	C	T	C	T
Baseline	3·5	3·5	9·8	9·8		
20	5·2	4·8•	39·1	25·3	21·3	26·5
40	6·9	6·1••	68·3	50·5†	49·3	57·3
60	7·3	6·8	97·7	65·5•••	70·8	81·2
80	5·9	5·5	93·2	60·8•••	75·2	81·7
100	4·5	4·2	61·6	36·6•••	67·5	77·2
120	4·0	3·9	63·7	31·7	64·8	69·2
150	4·5	3·7†	66·7	24·3†	57·2	66·5
180	4·4	3·6†	57·5	19·8†	53·2	55·2
210	3·7	3·4	47·8	16·7•	42·3	45·7
240	3·4	3·5	30·5	15·7	36·2	43·8••
270	3·2	3·7	22·3	18·3	28·7	36·7•
300	3·7	3·7	14·4	12·4	20·2	27·0•

C, control; T, test.
Significance of difference between test and control, •$P<0.05$, ••$P<0.025$, •••$P<0.01$, †$P<0.005$.

Jenkins, D. J. A., Leeds, A. R., Gussell, M. A. (1977). *Proc. Nutr. Soc.* **36**, 44A.
O'Keefe, S. J. D., Marks, V. (1977). *Lancet* i, 1286.

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Retinyl esters, retinol, retinol-binding protein and prealbumin in malnourished, xerophthalmic children treated by injection of vitamin A. By P. ANBUNATHAN and A. PIRIE, *Nutrition Rehabilitation Centre, Government Erskine Hospital, Madurai, India and Nuffield Laboratory of Ophthalmology, Walton Street, Oxford OX2 6AW*

When water miscible preparations of vitamin A are injected there is a rapid rise of serum vitamin, which is greatest and most prolonged in children with kwashiorkor (Pereira *et al.* 1967). Smith & Goodman (1976) have suggested that excess retinyl esters in serum, not bound to retinol-binding protein (RBP), may be a toxic factor in hypervitaminosis A. We have therefore studied the retinyl esters, retinol RBP and prealbumin (PA) in serum of severely malnourished, xerophthalmic children under treatment. Samples were taken before the first and 24 h after the last dose. A good local vegetarian diet was provided.

We first studied thirty-six children who were given between 150 000–300 000 I.U. retinyl palmitate (equivalent to 45–90 mg retinol) in water miscible form by intramuscular injection during 2–3 d. We calculated the molar retinol:RBP, normally near unity and also that of ester–retinol:RBP. The children were divided arbitrarily into those (twenty) with an ester–retinol:RBP value of 5 or more (mean 12·7) and those (sixteen) with a lower value (mean 2·6). Those children with the higher value had a significantly higher serum retinol and a significantly smaller increase in serum RBP and PA than those with a lower value.

Of thirteen children given 200 000 I.U. vitamin A (equivalent to 60 mg retinol), half by injection and half orally, (WHO, 1976) five developed an ester–retinol:RBP value of over 5 but increase of RBP and PA was the same in both groups.

Lack of protein can induce vitamin A deficiency. Vitamin A (Gopalan *et al.* 1960) and RBP in blood (Smith *et al.* 1973) may increase after feeds of protein devoid of vitamin A. Possibly protein synthesis was particularly impaired in those children who increased their serum RBP and PA least after dosage and had the highest value for ester–retinol:RBP. High serum levels of ester–retinol and of retinol not bound to RBP are unphysiological, possibly toxic and probably avoidable with lower initial dosage with vitamin A. The importance of good, protein-rich food as a part of treatment should be stressed.

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Acetate entry rates into portal and peripheral blood in the rabbit. By G. WOODNUTT and D. S. PARKER, *Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG6 2AJ*

Acetate present in the blood of ruminant animals is derived from two sources, the exogenous supply from the gut and the endogenous component resulting from tissue metabolism (Annison & Armstrong, 1970). In order to investigate the production of acetate from these two sources in the non-ruminant herbivore the entry rates of acetate in the fed and starved rabbit were determined. [U-^{14}C]acetate was infused for 3 h into the jugular vein of New Zealand White rabbits at a rate of 1 μCi/min. During the final hour of the infusion blood was collected from the carotid artery of the lactating animals and the carotid artery and portal vein of the non-lactating animals. Acetate concentration and specific radioactivity were determined by radio-gas-liquid chromatography and entry rates into carotid and portal blood calculated from a knowledge of infusion rate and plateau specific radioactivity. Infusions were carried out on animals that were fed *ad lib.* and also on animals starved for 18 h prior to the infusion. The results obtained are shown in the Table.

Acetate concentration (mM) and entry rate (μmol/min per kg) in fed, fasted and lactating New Zealand White rabbits

(Mean values with their standard errors, no. of animals in parentheses)

	Acetate concentration						Acetate entry rate					
	Fed			Starved			Fed			Starved		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
Carotid artery (non-lactating)	0·39	0·02	(81)	0·18	0·01	*(33)	20·9	2·5	(10)	9·1	1·7	*(4)
Portal vein (non-lactating)	0·81	0·05	†(57)	0·31	0·30	*‡(24)	65·5	11·2	†(4)	21·9	0·8	*‡(3)
Carotid artery (lactating)	0·54	0·02	†(31)	0·21	0·01	*(23)	34·0	1·8	†(8)	11·8	1·4	*(3)

*Significantly different from fed; $P<0.01$.
†Significantly different from carotid artery (fed); $P<0.01$.
‡Significantly different from carotid artery (starved); $P<0.02$.

Acetate entry rate into the carotid artery of fed lactating rabbits is significantly higher than in fed non-lactating rabbits, whereas when starved, the entry rates are not significantly different. This indicates that the increased acetate concentration in the carotid artery of lactating rabbits is derived from an increase in exogenous supply. Portal blood levels and entry rates of acetate show that considerable amounts of acetate are absorbed from the alimentary tract and that this supply is not entirely abolished by 18 h starvation, although the entry rate is significantly reduced.

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Soya-bean products in feeds for preruminant calves. By J. W. SISSONS and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Milk fed calves were fitted with abomasal and re-entrant ileal cannulas at 3 weeks of age. From about 5–7 weeks they were given single synthetic feeds containing heated fat-extracted soya flour (Product B, Smith & Sissons, 1975) as the sole protein source at 2–3 d intervals. By then they showed the digestive disorders, probably attributable to a gastro-intestinal allergy, reported previously (Sissons & Smith, 1976). Single synthetic feeds were then given at 2–3 d intervals in which product B was sometimes replaced by materials prepared by (a) extracting one part fat-free raw soya meal with four parts aqueous ethanol (75% v/v) at 78–80° for 2 h (Product K), (b) treating the meal under the same conditions but evaporating off the ethanol (Product L) or (c) a similar treatment but with one part soya meal to one part aqueous ethanol (Product M). Products K, L, M were steamed for 30 min and finely ground. Feeds prepared from a commercial alcohol extracted concentrate (Product D) (Danpro A, Arrhus Oliefabric A/S, Denmark) were also examined. The experiment was planned for each calf to receive all the feeds in each of two successive periods arranged in latin square designs. Mean values for transit time through the small intestine, mean ileal flow rates during 3 h and 21 h periods after the arrival of feed residues at the distal ileum and net N absorption up to this site were:

	Transit time (h)	Ileal flow rate (g/h) 3h	Ileal flow rate (g/h) 21 h	Net N absorption (proportion of intake)
Product B	1·66	502	152	44
D	2·50	268	87	66
K	2·59	204	76	73
L	2·11	283	105	66
M	2·05	201	114	61
SED (df)	0·22 (20)	117 (20)	22 (18)	8 (18)
5% LSD	0·45	245	45	16

Measurements after feeds prepared from casein were given at the beginning and end of the experiments were closely similar to those reported previously (Sissons & Smith, 1976).

Results confirmed the beneficial effects for calf feeding of extracting soya meal with hot aqueous ethanol and further indicated that at least part of these effects were due to the destruction of a toxic (probably allergenic) constituent in the flour rather than its extraction. This suggests the possibility of greatly improving soya products for calf feeding by a commercial ethanol treatment which would be much simpler than ethanol extraction.

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Metabolic abnormalities in patients prior to parenteral feeding. By D. J. NEWTON, R. G. CLARK, H. F. WOODS and H. CONNOR, (Introduced by R. J. CLARK), *Department of Therapeutics, Hallamshire Hospital and Department of Surgery, Northern General Hospital, Sheffield*

Many metabolic complications occur during intravenous feeding (Dudrick *et al.* 1972), and some of these are probably related to abnormalities that are present before parenteral nutrition is started (Woods & Marston, 1977).

We have studied twenty patients before feeding was begun. Diagnoses were; gastrointestinal carcinoma (9), inflammatory bowel disease (4), peptic ulceration (2), biliary fistulae (2) and other diseases (3). A control group of nineteen well-nourished patients was also studied.

Anthropometric measurements (weight, skinfold thickness at four sites, and mid upper arm circumference) were used to calculate arm muscle circumference and fat (% of total body-weight) by the methods of Durnin & Rahaman (1967). Venous blood samples were taken to estimate the concentrations of serum proteins, amino acids and intermediary metabolites of carbohydrate and fat metabolism. Haematological and vitamin tests were also performed.

Some of the differences between the two groups are summarized in the Table. No significant correlations were found between the anthropometric and biochemical measurements.

Comparison of measurements between well-nourished and malnourished patients

	Statistical significance of difference between groups*		Statistical significance of difference between groups
Serum albumin concentration	<0·001	Serum folate concentration	<0·05
Serum transferrin concentration	<0·01	Lymphocyte count	<0·01
Serum cholesterol concentration	<0·001	Haemoglobin concentration	<0·001
Blood lactate concentration	<0·05	Arm muscle circumference	<0·01
Leucocyte ascorbic acid concentration	<0·01	Fat (% of body-weight)	<0·01

*Mann-Whitney test.

These results show that the metabolic abnormalities in patients before the start of parenteral nutrition are complex and diverse. Thus 45% of patients had increased blood lactate concentrations, and 60% had low serum folate concentrations.

The lack of correlation between anthropometric and biochemical measurements suggests that the metabolic abnormalities in malnourished patients cannot be predicted by clinical examination.

D.J.N. is a Travenol Research Fellow.

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A comparison of several methods for assessing body fat content in mice.

By G. P. WEBB and P. D. ROGERS, *Department of Paramedical Sciences, North East London Polytechnic, London E*15 4*LZ*

Since measurement of body fat in the mouse by extraction procedures is time consuming and destructive, several alternatives were tested to assess their use in estimating body fat content in a normal population of forty-seven T/O mice.

The Lee index (Lee, 1928), whole body density and skinfold caliper measurements did not significantly correlate with percentage body fat and were thus found to be unsuitable. However, a highly significant correlation was found between the proportional weight of the gonadal fat pad and the percentage body fat (r 0·96 female, 0·82 male and 0·87 total). Furthermore, despite the differences in innervation and blood supply of the epididymal and parametrial fat pads, there was no significant difference between the slope or position of the two regression lines suggesting that fat is laid down in the same proportion in the gonadal fat pad of the two sexes. Extrapolation of the pooled regression line to the body fat content when there is no fat pad suggests that structural fat comprises an average 2.7% of the body-weight; any fat in excess of this is storage fat and is linearly deposited in the various fat organs.

The relationship reported here between gonadal fat pad and total body fat is similar to that found by Hull (1960) between abdominal fat and total fat but contrasts to the logarithmic relationship found by Liebelt *et al.* (1965) between extracted fat pad fat and total body fat. The gonadal organ has the advantage that it is readily accessible and discreet and since its weight is linearly related to body fat it can easily be used for a quantitative as well as a qualitative estimate; Lidiker (1966) previously used the size of storage fat organs, estimated on a scoring basis, to indicate the nutritive state of feral mice.

There was also a highly significant correlation between proportional fat pad weight and percentage body water (r −0·93). This would be expected in view of the well known correlation between body fat and body water (r −0·91).

Thus measurement of gonadal fat pad weight would be the most appropriate way to determine body fat or water in experimental situations where this information is required but where direct measurements are inappropriate.

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Transfer of elaidic acid (18:1 Δ 9-*trans*) across the rat placenta. By T. A. B. SANDERS, C. R. SOMAN and D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

It has been suggested that *trans* fatty acids, derived mainly from dairy fats and margarine, are undesirable constituents of human diets (Kummerow, 1975). When *trans* fatty acids were incorporated in the diet of rats, the metabolism of the essential fatty acid linoleic acid was partially inhibited (Privett & Blank, 1964; Anderson *et al.* 1975). The extent to which *trans* fatty acids may cross the placenta and thus influence foetal metabolism has not been investigated.

The dietary *trans* fatty acids are principally positioned isomers of *trans* octadecenoic acid, the major isomer being elaidic acid (18:1 Δ 9-*trans*). We decided therefore to analyse the liver lipids of foetuses from rats fed on a diet containing elaidic acid at a concentration found in human diets.

Six adult rats were mated then transferred from stock diet to an experimental diet containing 200 g fat/kg in which elaidic acid contributed 21% of the total fatty acids. Six littermate control rats received a similar diet in which elaidic acid was replaced with oleic acid. On day 21 of pregnancy the rats were killed and their foetuses were dissected. No difference was found between the food intakes of the two groups of rats, and the mean number of pups/litter, and weights of the pups and placentas were similar.

Fatty acid composition of total liver lipids from foetuses of dams fed on oleate or elaidate (mg/g total methyl esters detected)

(Mean values with their standard errors)

		16:0	16:1	18:0	18:1 *trans*	18:1 cis	18:2	20:4ω6	22:6ω3
Oleate	Mean	234	37	140	—	209	110	117	82
	SE	1·7	2·9	3·0	—	4·0	3·6	3·5	2·8
Elaidate	Mean	218*	47*	137	25**	203	106	120	87
	SE	1·5	1·1	2·4	2·3	6·2	3·0	1·6	4·1

*$P<0.05$; **$P<0.01$.

Elaidic acid was present in the liver lipids of the pups from dams given this fatty acid. The concentration was approximately one-tenth of that in the dietary fat. Incorporation of elaidic acid in the mothers' diet did not, however, appear to interfere with the transport of the polyunsaturated fatty acids across the placenta, or with the conversion of linoleic acid (18:2ω6) and linolenic acid (18:3ω3) to their major long-chain derivatives.

We gratefully acknowledge a grant from the Rank Prize Funds.

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An evaluation of the impact of breast feeding promotion in Edinburgh.

By T. R. KIRK, *Queen Margaret College, Edinburgh*

In 1974, the Department of Health and Social Security published a report which decried the low incidence of breast feeding in the United Kingdom and recommended that all mothers be encouraged to breast feed for 4–5 months. In the years following this report the advantages of breast feeding were much publicized in the media and by approximately mid-1976 it became the accepted policy of the medical and nursing professions to encourage breast feeding. To evaluate the effect of the subsequent breast feeding education, two surveys were conducted in Edinburgh which monitored the incidence and duration of breast feeding in mothers of infants born in the 12 months April 1974 to March 1975 and the 12 months October 1976 to September 1977, i.e. 30 months later. The samples were drawn from attenders at the same four Child Welfare Clinics. Mothers were interviewed either by T.R.K. or one of a team of dietitians and responses were recorded on standard questionnaires.

Table 1 shows the incidence of breast feeding following birth and at two weeks, 1 month and 4 months in both samples. Clearly a substantial increase in breast feeding occurred in the 30 months between the two study years. Inspection of the drop-out rate indicated, however, that the proportion of breast feeders introducing bottle feeding within 1 month did not change.

Table 1. *Breast feeding* (%) *in* 1974–75 *and* 1976–77

Breast-fed	1974–75 (*n* 78)	1976–77 (*n* 200)	Increase (%)
Initially	43·6	68·5	24·9
At 2 weeks	29·5	49·0	19·5
At 1 month	26·9	43·5	16·9
At 4 months	10·3	37·0	26·7

$P < .001$.

Further analysis revealed that the magnitude of the increase was greater in primiparous than multiparous mothers and varied with hospital of delivery. Moreover, there was a direct relationship between the magnitude of the increase and socio-economic status assessed by husband's occupation and even more clearly socio-economic status assessed by mother's–father's occupation. Thus it appeared that the breast feeding education instigated by the DHSS report was not uniformly effective throughout the Edinburgh population. In particular, it appeared to be relatively ineffective with mothers of low socio-economic status. Research is needed into methods of promoting breast feeding in these groups. In general, more attention should be given to the evaluation of currently used Nutrition Education methods and strategies.

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Dietary saponins and plasma cholesterol. By R. L. HOOD, D. G. OAKENFULL and D. L. TOPPING, *CSIRO Divisions of Food Research, North Ryde, New South Wales and Human Nutrition, Adelaide, South Australia*

Saponins are surface active sterol or triterpene glycosides which occur in a number of food plants, particularly soya beans, chick peas, peanuts and lucerne. Dietary saponins and foods rich in saponins have been known for many years to lower plasma cholesterol concentrations in animals (Newman, *et al.* 1957; Griminger, *et al.* 1958; Malinow, *et al.* 1977). A general explanation for the cholesterol-lowering action of many foods of plant origin is that bile acids are adsorbed by the plant fibre. This increases the loss of bile acids by excretion and the loss is offset by an increased conversion of cholesterol into bile acids by the liver (Kritchevsky, 1977). It has recently been shown that the presence of saponins is required for bile acids to adsorb to plant fibre preparations in vitro (Oakenfull & Fenwick, 1978). We have therefore examined the effects of diets containing 1% saponins on plasma lipids and faecal bile acids in the rat and pig.

In rats, dietary saponins increased the faecal excretion of bile acids and neutral sterols when the animals were on diets both low in cholesterol and high (1%) in cholesterol. Saponins also partially reversed the hypercholesterolaemia caused by the high cholesterol diet, as shown in the Table.

Plasma total cholesterol concentrations (*mg*/100*ml*)

(Mean values with their standard errors; five rats/group)

Standard synthetic diet	Diet +1% saponins	Diet +1% cholesterol	Diet +1% cholesterol +1% saponins
59 ± 3	60 ± 1	102 ± 3	80 ± 4

In pigs on a low fat, low cholesterol diet, dietary saponins lowered plasma cholesterol and increased faecal excretion of bile acids and neutral sterols. In both species the contribution of primary bile acids (particularly chenodeoxycholic) to faecal excretion was increased by saponins.

Our results are consistent with the hypothesis that dietary saponins induce adsorption of bile acids by fibre. Saponins are present in most of those food plants and plant products which have been shown to lower plasma cholesterol concentrations in man or experimental animals whereas wheat bran, which is free from saponins, has no effect (Truswell & Kay, 1976). Thus the hypocholesterolaemic effect of foods (such as soya-bean products) which are rich in saponins appears to be a consequence of their high saponin content.

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