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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Eighteenth Meeting of the Nutrition Society (One Hundred and Sixty-sixth of the Scottish Group) was held in the Dunfermline College of Physical Education, Cramond, Edinburgh on Tuesday and Wednesday, 17/18 *September* 1985, *when the following papers were read:*

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Effects of drugs altering arachidonic acid metabolism on production of interleukin 1 by monocyte-like cells. By D. SHAPIRO, J. J. F. BELCH, R. D. STURROCK and A. SHENKIN, *Department of Pathological Biochemistry and Medicine, Royal Infirmary, Glasgow*

Interleukin 1 (IL1) is an inflammatory hormone released by macrophages and epithelial cells. Apart from its properties of causing fever, inducing the acute-phase response to injury and stimulating the immune system, it has also been reported to cause protein degradation in skeletal muscle. This may explain the increased net protein catabolism which occurs after injury and during infections. It is of interest to explore whether it is possible to modulate IL1 production pharmacologically.

It has been suggested that compounds which affect arachidonic acid metabolism are capable of altering IL1 release (Kunkel & Chensue, 1985). We have further investigated this effect by incubating a human promonocytic cell line, U937, with a stimulus for IL1 release and various compounds which affect production of prostaglandins or leukotrienes. The drugs used were: indomethacin, a cyclooxygenase inhibitor; BW755C, a combined cyclo- and lipoxygenase inhibitor; Dazmegrel, a thromboxane synthetase inhibitor; iloprost, a prostacyclin analogue; and prostacyclin. U937 cells were preincubated with the drug at various concentrations for 1 h, toxic shock syndrome toxin derived from *Staphylococcus aureus* was added to stimulate IL1 production, and the incubation was continued for 24 h. The medium was dialysed and samples were passed through an ultrafiltration membrane with a molecular weight cut-off of 30 000 daltons to remove interfering substances. IL1 was assayed by incorporation of [^{3}H]thymidine into murine thymocytes.

Drug (μg/ml or ng/ml) and IL1 (U/l) concentrations (n 6)

Control IL1		Indomethacin			BW755C			Dazmegrel			Iloprost			Prostacyclin		
		Drug	IL1		Drug	IL1		Drug	IL1		Drug	IL1		Drug	IL1	
Mean	SD	(μg/ml)	Mean	SD	(μg/ml)	Mean	SD	(μg/ml)	Mean	SD	(μg/ml)	Mean	SD	(μg/ml)	Mean	SD
95	24	1	89	7	50	98	13	1	114	16	1	122*	17	1	131*	35
(*n* 12)		10	92	15	100	86	28	5	43***	11	10	159***	39	10	63*	19
								10	23***	16	50	80	9	50	89	17

Significantly different from control value (*t* test): *$P<0.05$; ***$P<0.001$.

When cyclooxygenase alone or both cyclooxygenase and lipoxygenase were inhibited, IL1 was not affected. However, with the combined inhibitor only, the assay dilution curve of unfiltered samples was very shallow, indicating substantial release of an IL1 inhibitor. This suggests that leukotrienes may control synthesis of this inhibitor. Inhibition of thromboxane synthetase markedly reduced IL1 release. This may not be a direct effect of thromboxane, since Dazmegrel is known to cause increases in other prostaglandins, at least one of which, PGE_2, inhibits IL1 release. Prostacyclin augmented IL1 release: its lack of effect at high doses may reflect feedback inhibition of prostanoid synthesis. The relevance of these observations to control of protein metabolism in disease requires further study.

Kunkel, S. L. & Chensue, S. W. (1985). *Biochemical and Biophysical Research Communications* **128**, 892–897.

Associations between feeding, housing and the incidence of abomasal ulcers in veal calves. By D. DE B. WELCHMAN, *Department of Animal Husbandry, University of Bristol, School of Veterinary Science, Langford, Bristol BS18 7DU* and GUL N. BAUST, *Food Research Institute, Langford, Bristol BS18 7DY* (Introduced by A. J. F. WEBSTER)

When calves are reared for veal on liquid diets, similar in composition to cow's milk, and slaughtered at an age of 15–18 weeks, it is common to see multiple small erosions and ulcers predominantly in the pyloric region of the abomasum. Calves reared on conventional diets are not killed at this age so it is not known if the lesions are a consequence of the veal system and of a severity sufficient to impair growth and feed conversion. Van Putten (1982) observed that lesions increased when veal calves were housed on straw. In the UK, veal calves are commonly reared in groups either on straw bedding or wood shavings (with access to straw) with milk replacer provided *ad lib.* via teats from an automatic dispenser.

Post mortem examinations were performed on the abomasa of 227 clinically healthy veal calves of known provenance at commercial slaughterhouses. The incidence of pyloric lesions in calves kept in wooden crates and given two liquid feeds daily (bucket-fed) was 41/62 (system A). For calves offered milk replacer *ad lib.* and given access to barley straw (system B), the incidence was 68/69 for those on straw bedding and 91/96 for those bedded on wood shavings. The difference between systems was significant (χ^2 45·0, $P<0{\cdot}001$). Calves were scored for incidence and severity of lesions: high scores were not associated with reduced growth rate. The incidence of pyloric lesions in 132 calves reared for veal in five different experimental regimes at the University of Bristol is given in the Table.

System	Experimental regime	Incidence
1.	Calves in crates, milk-fed twice daily, no dry food	8/11
2.	Calves in crates, milk-fed twice daily, plus digestible dry food	37/38
3.	Calves on straw, milk-fed twice daily	15/18
4.	Calves on straw, milk-fed *ad lib.* via teats	35/37
5.	Calves on straw, computer-rationed milk feeding via teats plus digestible dry food	22/28

Systems 1 and 4 correspond to commercial systems A and B respectively. System 3 corresponds to Van Putten's (1982) veal calves on straw. These observations confirm that the incidence of pyloric lesions is increased when veal calves are also given access to straw. The lesions do not, however, appear to affect performance or present clinical signs of ill-health. Lesions may be reduced by restricting milk intake and dividing it between a large number of small meals (system 5).

David de B. Welchman acknowledges support from the Animal Health Trust.

Van Putten, G. (1982). *Veterinary Record* **111**, 437–440.

A study of the time course of fructose-2,6-bisphosphate production in a septic mouse model. By D. HEPBURN, J. BROOM and D. J. SMITH, *Surgical Metabolic Unit, Department of Surgery, University of Aberdeen, Foresterhill, Aberdeen AB9 2ZB*

Where sepsis occurs in the post-surgical period, mortality rates tend to be high. In sepsis, metabolism is grossly disrupted with glucose becoming the preferred fuel for energy provision even where ketogenesis has been promoted. Glycogen stores are rapidly exhausted and body protein is wasted through proteolysis which serves to provide the gluconeogenic precursors for glucose formation in the liver (Imamura *et al.* 1975). Ketone bodies disappear from the circulation whereas circulating glucose levels are elevated and glycolytic flux is increased. This increase is thought to be strongly influenced by the recently discovered metabolite, fructose-2,6-bisphosphate (F2,6P2), which is the most effective accelerator of phosphofructokinase activity known to date (Van Shaftingen *et al.* 1980). High levels of F2,6P2 are present in the liver in the fed state whereas levels are very low in the fasted state. A previous study using a septic mouse model showed that the hitherto low levels of F2,6P2 in fasting mouse livers were greatly increased within 3 h of the septic insult (Hepburn & Broom, 1985): no such increase was noted in the fed septic animals where levels remained high despite a marked depression of glycogen stores.

The present study investigated the time course of F2,6P2 increase after inoculation with live *Escherichia coli*. Using the same septic model, control and septic fasted animals were killed at 15, 30, 60, 90 and 180 min post-inoculation and livers were rapidly freeze-clamped. Little difference between fasted control and septic animals was observed at 15 or 30 min (0·751 (SD 0·185) and 0·740 (SD 0·207) pmol/g) but by 60 min F2,6P2 levels had virtually trebled (1·763 (SD 0·478) pmol/g) and production continued to increase between 90 and 180 min post-inoculation (1·922 (SD 0·092) and 3·713 (SD 0·280) pmol/g). Thus sepsis elevated F2,6P2 production within 1 h of inoculation in contrast with the results of a study by Kuwajima *et al.* (1984) on fasting and refeeding of healthy mice where a delay of 6 h was observed before elevation of F2,6P2 levels in the liver.

A differing time scale of events occurs in sepsis compared with that of the normal state and this may be governed by activation of an existing enzyme or by *de novo* synthesis of 6-phosphofructose-2-kinase, the enzyme involved in F2,6P2 production. Sepsis might remove inhibition of enzyme activity in the fasted state, thus increasing F2,6P2 levels, whereas refeeding is perhaps associated either with *de novo* synthesis of the enzyme or with a very slow derepression of enzyme activity.

Hepburn, D. & Broom, J. (1985). *Clinical Nutrition*, Suppl. (In the Press).
Imamura, M., Clowes, G. A. A., Blackburn, G. L. & O'Donnell, T. F. (1975). *Surgery* **77**, 868.
Kuwajima, M., Newgard, C. B., Foster, D. W. & McGarry, J. D. (1984). *Journal of Clinical Investigation* **74**, 1108–1111.
Van Shaftingen, E., Hue, L. & Hers, M. G. (1980). *Biochemical Journal* **192**, 887–895.

Further observations on the effects of supplements of iron on growing fowl inoculated with *Salmonella gallinarum*. By I. M. SMITH and R. HILL, *Royal Veterinary College, Boltons Park, Potters Bar, Herts*

In earlier experiments the survival of male layer-type hybrid fowl inoculated orally with *Salmonella gallinarum* was greater in those given a diet based on meat meal (16B) than in those given a similar diet in which fish meal (16F) replaced meat meal (Hill & Smith, 1977); also, the inclusion of FeEDTA in a mixed protein diet (MPD) increased survival time (Smith *et al.* 1977).

We now report an interaction between the basal diet and FeEDTA. The experiments were carried out as described earlier (Smith *et al.* 1977), chicks being reared from 1 to 12 d of age on a commercial type diet and given the experimental diets thereafter. At 15 d the birds were inoculated orally with a suspension of *S. gallinarum* and survival was recorded 18 d later. Groups were given basal (MPD), meat (16B) and fish (16F) diets in each case with 0, 200 or 400 mg Fe/kg added as FeEDTA. The numbers of birds surviving are given in the Table.

FeEDTA (mg/kg)	Diet ...	Survival % (of 65 per treatment) MPD	16F	16B
0		14	15	26
200		45	22	36
400		60	18	54

A marked increase in survival occurred from the addition of FeEDTA to meat and mixed protein diets, but not to the fish diet.

The above experiment and those described earlier were all carried out using one strain of *S. gallinarum* S.G.9. Five other strains have been tested using the mixed protein diet, with and without the addition of 400 mg Fe/kg as FeEDTA, with the following results.

Diet	SG strain ...	Survival % (of 20 per treatment) 9	373	383	5441	7285	Blandford
MPD		35	20	70	10	20	85
MPD + FeEDTA		55	50	95	40	30	100

With all six strains survival was increased by the inclusion of FeEDTA and the improvement was similar for strains of high or low virulence.

These results support those of earlier experiments in showing increased resistance of growing fowl against *S. gallinarum* when given a dietary supplement of FeEDTA, and the protective effect occurred with a wide range of strains of *S. gallinarum*. However, the high susceptibility with a fish-meal diet was not altered by the addition of FeEDTA.

Hill, R. & Smith, I. M. (1977). *British Journal of Nutrition* **38**, 471–478.
Smith, I.M., Hill, R. & Licence, S. T. (1977). *Research in Veterinary Science* **23**, 263–268.

Measles, energy balance and childhood growth. By M. B. DUGGAN and R. D. G. MILNER, *Department of Paediatrics, University of Sheffield, Children's Hospital, Western Bank, Sheffield S10*

The magnitude of negative energy balance in a childhood infection and its nutritional implications were quantified during a study of twenty black Kenyan children admitted to hospital with acute measles. The energy contents of a 24 h duplicate food intake and a 24 h collection of faeces and urine were determined experimentally. The metabolic rate was determined by flow-over indirect calorimetry. An identical control study was carried out after monitoring convalescence at home. Full anthropometry was carried out in hospital. Apparent energy balance (EB) was estimated as the difference between the metabolizable energy intake (MEI) and the energy expenditure (EE) during resting metabolism.

The mean 24 h MEI during measles represented 25% of the mean MEI in the control study, while mean EE was similar in the two studies. The mean level of EB was 67 (SEM 30·0) kJ/kg per 24 h in controls; during measles it was −169 (SEM 21·7) kJ/kg per 24 h. The data were also used to estimate the maintenance energy requirement. In controls, the level of postprandial metabolism was 13% greater than the resting rate; this significant ($P<0·05$) difference was absent during measles, when the postprandial metabolic rate was marginally lower than the resting rate. During convalescence there was a significant improvement in mean weight/age and weight/length and a significant deterioration in mean length/age standard deviation score ($P<0·01$ in each case, paired t test).

Measles is characterized by a fall in energy intake, a sustained level of energy expenditure and no postprandial enhancement of the metabolic rate. During convalescence satisfactory weight gain is accompanied by faltering in linear growth.

The effect of fasting on the acute response of protein synthesis in muscle and liver to the *Escherichia coli* lipopolysaccharide. By M. JEPSON, J. M. PELL and D. J. MILLWARD, *Nutrition Research Unit, London School of Hygiene and Tropical Medicine, 4 St Pancras Way, London NW1 2PE*

In well-fed rats treated with the *Escherichia coli* lipopolysaccharide endotoxin (LPS), the catabolic response of muscle reflects decreased protein synthesis and increased proteolysis, while in the liver protein synthesis is increased as part of the acute phase response (Jepson *et al.* 1985). Because of the sensitivity of muscle protein synthesis to changes in food intake the decreased protein synthesis could be an indirect response since exposure to LPS depresses food intake, although this is not marked and insulin levels are not depressed. We have therefore evaluated the response to LPS in the fasted state. This also allows investigation of the dependency of the increased hepatic protein synthesis on dietary protein.

Food was removed from four groups of six young male rats. Two groups were injected with the LPS (3 mg/kg, subcutaneously) and two groups with saline (9 g sodium chloride/l). After 24 h, one treated and one control group were killed 15 min after the injection of a large dose of [^{3}H]phenylalanine and protein synthesis measured in muscle and liver. The other two groups were treated with a second dose of LPS or saline and killed at 30 h with a similar measurement of protein synthesis. Plasma insulin levels were also measured and the results are shown in the Table together with the results of our previous measurements in well-fed rats.

	Muscle protein synthesis				Liver protein					
	(%/d)		(g protein/g RNA per d)		Content (g/kg initial wt)		Synthesis (%/d)		Insulin (units/ml)	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control fed	17·5	0·5	11·3	1·1	6·64	0·60	111	15	31	5
+LPS 24 h	10·3	1·7	6·9	1·2	7·25	0·48	149	21	34	7
+LPS 30 h	8·4	0·8	5·8	1·1	7·45	0·72	142	8	32	5
Control fasted 24 h	11·3	1·9	9·7	1·4	5·78	0·32	106	26	17	9
+LPS 24 h	8·5	0·9	7·9	0·7	6·65	0·41	130	23	30	10
Control fasted 30 h	8·9	1·3	8·2	1·2	5·74	0·36	100	11	17	0·5
+LPS 30 h	7·9	1·2	6·6	0·8	6·63	0·35	122	14	31	14

Muscle protein synthesis was depressed by the LPS treatment of the fasted rats ($P<0·01$) even though insulin concentrations increased ($P<0·05$), demonstrating that this response is independent of changes in food intake and involves a complete insulin resistance of muscle protein synthesis. Hepatic protein synthesis was still increased in the fasted state and since the fasting-induced loss of liver protein was prevented it is clear that extrahepatic tissues must have provided sufficient amino acids to allow the acute phase response and balance export protein synthesis.

Jepson, M. M., Pell, J. M., Bates, P. C. & Millward, D. J. (1985). *Biochemical Society Transactions* (In the Press).

The effect of guar gum on the mucosal regulation of iron absorption in rats. By T. E. SWINDELL and I. T. JOHNSON, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA*

A regulatory effect of previous iron intake on the subsequent retention of Fe from test meals has been reported in rats (Fairweather-Tait *et al.* 1985) and it may be exerted at the intestinal mucosa. The effect of guar gum on the regulation of Fe absorption was investigated because it is known to alter both mucosal cell proliferation and enzyme and carrier activity in the proximal small intestine (Johnson *et al.* 1984).

Male Wistar rats (80–100 g) were randomly allocated into two groups. Both received semi-synthetic diets, one containing 40 g cellulose/kg (C), the other additionally containing 100 g guar gum/kg (G), as their fibre components. The diets were given *ad lib.* for 14 d in the case of C-fed animals and 20 d in the case of G-fed animals, to ensure the attainment of similar body-weights. Both groups were then trained to meal-feed over a further 7 d, so that they consumed food for 1 h in any 24, and were then given either a low-Fe meal (8 μg/g) or a high-Fe meal (566 μg/g). At intervals after these meals the luminal loss of ^{59}Fe-labelled ferric citrate from duodenal loops, over a 30 min period, was determined in vivo. The results are shown in the Table.

Luminal loss of ^{59}Fe-labelled ferric citrate from G-fed animals and C-fed animals given low- or high-Fe test meals

		High-Fe test meal				Low-Fe test meal			
Time interval after test meal (h) ...		12	36	60	84	12	36	60	84
Luminal loss (μmol/g dry wt of duodenal loop) in:									
G-fed group:	Mean	0·416	0·317**	0·294	0·419**+	0·629***	0·501***	0·460*	0·478***
	SEM	0·025	0·021	0·030	0·065	0·092	0·067	0·04	0·038
C-fed group:	Mean	0·506‡	0·504‡	0·377‡	0·664	1·169+	1·076‡	0·615‡	0·664
	SEM	0·055	0·053	0·026	0·092	0·056	0·071	0·059	0·042

Significant difference between G-fed and C-fed groups: *$P<0·05$, **$P<0·01$, ***$P<0·001$.
Mean values for nine animals per group except where indicated: +n 8, ‡n 7.

The G diet had no significant effect on Fe status as measured by haemoglobin and total liver Fe levels. In both groups the low-Fe meal enhanced Fe absorption, whilst the high-Fe meal depressed it, this effect completely disappearing after 60 h in the case of low-Fe and 84 h in the case of high-Fe. The G-fed animals showed depressed levels of Fe absorption in comparison with C-fed animals. This effect of guar gum may be due to increased cellular proliferation, leading to a shorter villous transit time and a decrease in the average lifespan of the mucosal cells.

T. E. S. acknowledges receipts of an AFRC research studentship.

Fairweather-Tait, S. J., Swindell, T. E. & Wright, A. J. A. (1985). *British Journal of Nutrition* **54**, 79–86.
Johnson, I. T., Gee, J. M. & Mahoney, R. R. (1984). *British Journal of Nutrition* **52**, 477–487.

Effect of a lipoxygenase inhibitor, AA861, on the metabolic response to *Escherichia coli* endotoxin in rats. By JENNIFER WAN and R. F. GRIMBLE, *Nutrition Department, Southampton University Medical School, Southampton SO9 3TU*

Diets containing coconut oil (30 and 200 g/kg) abolish many of the responses to *Escherichia coli* endotoxin, i.e. a fall in serum zinc, elevation of corticosterone and loss of protein from muscle, skin and bone (Wan *et al.* 1986). Coconut oil could influence the responses via a reduction in prostaglandin or leukotriene production since the oil is poor in lineolate, the precursor of arachidonate, and ultimately of both types of eicosanoid. Inhibition of prostaglandin production with indomethacin abolishes the fever response to *E. coli* endotoxin but has no effect on the fall in serum Zn (Tocco *et al.* 1983). Thus not all endotoxin effects are mediated via prostaglandins and we investigated the role of leukotrienes by pretreatment of rats with a lipoxygenase inhibitor, AA861, before intraperitoneal (i.p.) injection of endotoxin.

Female Wistar rats (188±3 g) were assigned to five groups. Two received 37·5 mg AA861/kg (L) in carboxymethylcellulose (CMC; 50 g/kg) orally, one group a higher dose of 75 mg AA861/kg (H) in CMC, and two groups CMC alone. After 1 h, half the rats given AA861 (L), half the rats given CMC alone and the rats given AA861 (H) received *E. coli* endotoxin (400 μg/kg body-weight) i.p., the remaining rats received sterile saline (9 g sodium chloride/l). All rats were pair-fed on standard laboratory chow, to the amount eaten by the AA861 (H) group (6±1 g). Rats were killed 24 h after injection by decapitation, the blood collected, pelts removed and various tissues rapidly dissected and frozen in liquid nitrogen and all samples stored at −20°. Protein was measured by the Folin Ciocalteau method, serum Zn by atomic absorption spectrometry and corticosterone by radioimmunoassay.

Pretreatment (*n*) . . .	CMC (5)		CMC (4)		AA861 (H) (4)		AA861 (L) (4)		AA861 (L) (5)	
E. coli endotoxin . . .	−		+		+		+		−	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body-wt loss (g)	8	2	18*	1	20*	1	14	3	8	1
Pelt wt (g/kg body-wt)	174	2	165*	1	158*	3	169	6	166*	4
Liver total protein (g)	1·51	0·04	1·78*	0·06	2·29*†	0·08	1·85*	0·14	1·58	0·02
Protein concentration (g/kg):										
Muscle (thigh)	206	4	189*	3	196	4	197	3	206	4
Skin (abdomen)	158	3	140*	3	134*	7	149	5	149	8
Femur	109	1	91*	2	97*	4	90*	4	109	2
Serum Zn (μg/ml)	2·04	0·08	0·8*	0·05	1·85*†	0·07	1·42*†	0·17	2·01	0·03
Corticosterone (ng/ml)	96	17	628*	97	500*	66	495*	77	200*†	37

Significantly different from group receiving CMC alone: *$P<0·05$.
Significantly different from group receiving CMC and endotoxin: †$P<0·05$.

AA861 prevented the depression of serum Zn but had no clear-cut inhibitory effect on other endotoxin effects. Leukotrienes may mediate the depression of serum Zn in endotoxaemia.

The authors are grateful to Takeda Chemical Industries for the gift of AA861.

Tocco, R. J., Kahn, L. L., Kluger, M. J. & Vander, A. J. (1983). *American Journal of Physiology* **244**, R368–R373.
Wan, J., Grimble, R. F. & Gore, M. (1986). *Proceedings of the Nutrition Society* **45**, 27A.

The impact of zinc deficiency on the intestinal response to cholera toxin.

By S. ROY, B. S. DRASER and A. M. TOMKINS, *International Centre for Diarrhoea Disease Research, Bangladesh* and *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Severe diarrhoeal disease is a frequent complication of protein–energy malnourished (PEM) children in developing countries. Among the nutritional deficiencies associated with PEM, zinc deficiency is potentially important because of its impact on structure and function of the intestinal mucosa.

An in vivo intestinal perfusion system in female Sprague–Dawley rats was established using fluid containing sodium chloride, potassium chloride, glucose, sodium bicarbonate and polyethylene glycol 4000, some of which was labelled with ^{14}C (5 μCi/l).

Net transport of water and electrolytes in response to cholera toxin (CT) at different stages of experimental Zn-deficiency in female rats

Group	*n*	Perfusion fluid status	Net water transport (μl/mm intestine per h) Mean	SE	Net Na transport (mmol/mm intestine per h) Mean	SE	Net K transport (mmol/mm intestine per h) Mean	SE
Ad lib. fed control (ZAL)	5	No toxin	+4·85	0·58	+0·72	0·20	+0·007	0·02
Zinc deficient (ZD)	5	No toxin	+2·76	0·20	+0·35	0·11	+0·007	0·02
Zn deficient with 48 h repletion (ZDR)	4	No toxin	+5·77	0·52	+0·77	0·30	+0·003	0·01
Ad lib. fed control (ZAL)	5	0·1 μg CT/ml	−1·84	0·76	−0·20	0·045	−0·04	0·09
Zn deficient (ZD)	6	0·1 μg CT/ml	−5·50	0·89	−0·88	0·29	−0·08	0·016
Zn deficient with 48 h repletion (ZDR)	5	0·1 μg CT/ml	−3·30	1·29	−0·54	0·31	−0·056	0·013

Statistical significance of difference:
Water transport (no toxin): ZD *v.* ZAL $P<0·01$, ZD *v.* ZDL $P<0·01$; (with toxin): ZD *v.* ZAL $P<0·01$, ZD *v.* ZDR not significant.
Na transport (no toxin): ZD *v.* ZAL $P<0·05$, ZD *v.* ZDR $P<0·05$; (with toxin): ZD *v.* ZAL $P<0·01$, ZD *v.* ZDR not significant.
K transport: not significant.

Among animals given Zn *ad lib.*, there was net absorption of water, Na and K. Animals made Zn-deficient by feeding a diet containing <1 mg Zn/kg for 16 d showed impaired absorption of water and Na. When cholera toxin (1 μg/ml) was added to the perfusate the Zn-deficient animals showed a marked secretion of water, Na and K. A separate group of Zn-deficient animals received a diet containing 55 mg Zn/kg *ad lib.* for 48 h before the infusion. Their rates of secretion were less than in the non-repleted, Zn-deficient animals.

These results suggest that studies of the impact of Zn supplements on fluid and electrolyte loss in malnourished children with enteric diseases are necessary.

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The influence of human and bovine lactoferrin on iron absorption from ferrous sulphate in rats. By SUSAN J. FAIRWEATHER-TAIT, A. J. A. WRIGHT and ZOE PIPER, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA* and J. L. LEUBA, *Nestlé Research Dept., Nestec Ltd, Vevey, Switzerland*

Human milk contains considerably more lactoferrin (LF) than cow's milk and there is much speculation about the role of LF in infant nutrition. Apart from its possible involvement in modifying the gut microflora by binding Fe, the extent to which the LF-bound Fe can be absorbed by the infant is not yet clear; studies are currently underway to resolve this issue by feeding ^{58}Fe-labelled bovine LF to infants. The objective of the present investigation was to compare the effect of human and bovine LF on Fe absorption from a small amount of $FeSO_4$, using rats as a model for man, and to test whether the initial degree of Fe saturation influenced Fe absorption from bovine LF.

Adult male Wistar rats were given a semi-synthetic control diet (Fairweather-Tait *et al.* 1984) for 2 weeks. After an overnight fast the rats (300 g) were given 5 g cooked starch:sucrose (1:1) paste containing human (Sigma Chemical Co., Poole, Dorset) or bovine (Nestlé Research, Vevey, Switzerland) LF or $FeSO_4$, or both, as shown in the Table. Each meal was labelled with 0·5 μCi ^{59}Fe (Amersham International plc, Amersham, Bucks). The rats were counted for radioactivity in a small animal whole-body counter (Nuclear Enterprises, Edinburgh) immediately after consuming the meal and again 7 d later (Fairweather-Tait & Wright, 1984) and percentage Fe absorption calculated.

		Fe (μg)			% Fe absorbed	
Test material	Wt (mg)	Already present	Total Fe-binding capacity	Added as $FeSO_4$ to test meal	Mean	SEM
$FeSO_4 \cdot 7H_2O$	0·199	40·0	—	—	75·7	1·2
Human LF (11% saturated)	10	1·6	13·2	38·4	76·6	1·4
Bovine LF (14% saturated)	10	2·0	6·8	38·0	77·8	1·7
Desaturated bovine LF (7% saturated)	10	1·0	10·3	39·0	32·0***	2·5

***$P<0.001$.

The desaturated bovine LF was prepared by dialysing against 0·1 M-phosphate buffer containing EDTA (10 g/l) (Spik *et al.* 1978). This procedure increased the total Fe-binding capacity from 47 to 71% of theoretical, compared with human LF with a binding capacity of 91%. The desaturated LF significantly suppressed the absorption of Fe from $FeSO_4$ ($P<0.001$) but the human and untreated bovine LF had no effect.

Fairweather-Tait, S. J. & Wright, A. J. A. (1984). *British Journal of Nutrition* **51**, 185–191.
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The effect of extrusion cooking of maize on iron and zinc availability. By SUSAN J. FAIRWEATHER-TAIT and LISA L. SYMSS, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA*

Little is known about the nutritional implications of modern techniques of food processing such as extrusion cooking, which is used widely in the production of instant and snack foods, breakfast cereals and crispbreads. The availability of iron and zinc from extruded and non-extruded maize was therefore compared with ferrous sulphate and zinc chloride.

Ground, degermed maize kernels were extruded by the Solids Processing Group at the Food Research Institute, Norwich, in a Baker Perkins MPF 50 twin-screw extruder at 120–140° and 6·21 MPa (900 psi). Samples of both extruded and non-extruded maize were freeze-dried, ground to a fine powder and the Fe and Zn contents measured by atomic absorption spectroscopy.

Young male Wistar rats were trained to meal-feed on a control, semi-synthetic diet (Fairweather-Tait *et al.* 1984) for 2 weeks when their mean weight was 250 g. After an overnight fast they were given a test meal of extruded or non-extruded maize containing similar levels of Fe (20 μg) or Zn (20 μg) or 5 g cooked starch: sucrose (1:1) paste containing 20 μg Fe ($FeSO_4$) or 20 μg Zn ($ZnCl_2$) in 0·1 M-hydrochloric acid, extrinsically-labelled with 0·5 μCi ^{59}Fe ($FeCl_3$) or 1 μCi ^{65}Zn ($ZnCl_2$) (Amersham International plc, Amersham, Bucks). The rats were counted for radioactivity in a small animal whole-body γ-counter (NE 8112, Nuclear Enterprises, Edinburgh) immediately after consuming the meal and those given ^{59}Fe were counted 7 d later, as described previously (Fairweather-Tait & Wright, 1984), and the proportion of Fe absorbed from the test meal was calculated. The animals given ^{65}Zn were counted daily for a further 13 d. The rate of loss of ^{65}Zn was constant between days 5 and 13, once all the unabsorbed ^{65}Zn had been excreted. It was therefore possible to estimate true absorption on day 0 using regression analysis by plotting log% ^{65}Zn retention against time (days 5–13). The results are shown in the Table.

	% Fe absorbed			% Zn absorbed		
Test meal	No. of animals	Mean	SE	No. of animals	Mean	SE
$FeSO_4$ or $ZnCl_2$	16	66·6[a]	1·8	18	44·1[c]	1·0
Non-extruded maize	19	60·7[b]	1·5	19	53·9[d]	1·0
Extruded maize	13	66·0[ab]	3.2	12	60·7[e]	1·0

Mean values with different superscript letters are significantly different: [a,b]$P<0·01$, [c,d,e]$P<0·001$.

Extrusion cooking of maize appeared to significantly improve the availability of Zn, without altering Zn turnover. The effect on Fe availability was less clear; there was no significant difference in Fe absorption between the extruded and non-extruded maize, but Fe from the non-extruded maize was less well-absorbed than that from $FeSO_4$, which suggests a marginal improvement in Fe availability with extrusion cooking.

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The metabolism of *t*-butyl hydroperoxide and hydrogen peroxide by isolated perfused liver from control and selenium-deficient rainbow trout (*Salmo gairdneri*). By J. G. BELL, J. W. ADRON and C. B. COWEY, *NERC Institute of Marine Biochemistry, St Fittick's Road, Aberdeen AB1 3RA*

Peroxide removal in mammals is attributed to the peroxisomal enzyme catalase (*EC* 1.11.1.6) and the mitochondrial and cytosolic selenoenzyme glutathione peroxidase (*EC* 1.11.1.9; GSHPx). Both enzymes destroy hydrogen peroxide, and GSHPx is seen also as an important means of organic hydroperoxide removal. In addition, a selenium-independent GSHPx activity (due to one or more of the glutathione S-transferases (*EC* 2.5.1.18)) which removes organic hydroperoxides but not H_2O_2, has been demonstrated in rats under conditions of Se deficiency (Lawrence & Burk, 1976).

Trout liver GSH S-transferases do not exhibit GSH peroxidase activity (Bell *et al.* 1984). However, GSH S-transferase in both liver and plasma of Se-deficient fish is significantly increased over that in control fish (Bell *et al.* 1986) and GSH S-transferase purified from trout liver in microsomal lipids in vitro reduces malondialdehyde formation (Bell *et al.* 1984).

Release of oxidized glutathione (GSSG) in response to infusion of peroxide has been examined in the isolated perfused haemoglobin-free trout liver in a recirculating system. Addition of *t*-butyl hydroperoxide to this system led to a marked, extended release of GSSG into the perfusate. When H_2O_2 was infused, a similar increase in GSSG efflux was observed but in this case it reached a peak value about 20 min after H_2O_2 addition and decreased thereafter. This result may have been due to the metabolism of H_2O_2 by catalase. Thus, on addition of the catalase inhibitor 3-amino-1,2,4-triazole to the perfusing fluid, subsequent release of GSSG, on addition of H_2O_2, was similar to that obtained when *t*-butyl hydroperoxide alone was perfused.

The perfusion of Se-deficient trout liver with both *t*-butyl hydroperoxide and H_2O_2 led to a small release of GSSG similar to that obtained in livers of control trout where no hydroperoxide was added. These results indicate that any protective action against oxidation afforded by the enhanced levels of GSH S-transferase activity in Se-deficient trout does not function by increased GSSG formation, that is, it is not a Se-independent GSHPx.

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Exogenous supply of glucose precursors and nitrogen utilization in sheep.

By C. P. GIRDLER, P. C. THOMAS and D. G. CHAMBERLAIN, *Hannah Research Institute, Ayr KA6 5HL*

Information was sought on the minimum glucose requirement of sheep and on the competing demands for amino acids for protein synthesis and gluconeogenesis.

Four sheep (body-weight (W) 55·4±1·78 kg) were sustained for six 4–8 d periods by intraruminal infusions of acetate (Ac), propionate (Pr), butyrate (Bu), buffer and minerals and intraabomasal infusions of casein, emulsified tallow, glucose and vitamins (Table 1). Isoenergetic infusions provided 495 kJ ME/kg $W^{0.75}$ per d (1·1 times maintenance). In period 1 (P1), propionate but no glucose was given and the casein dose was set to give approximately zero N retention. Subsequent treatments involved withdrawal of all propionate (P2), replacement with glucose (P3, P4, P5) and withdrawal of glucose (P6).

Table 1. *N retention in sheep given intraruminal infusions of different proportions of acetate, propionate and butyrate and intraabomasal infusions of glucose, casein* (480 *mg N/kg* $W^{0.75}$ *per d*) *and tallow* (25 *g/d*). *Major minerals, buffer and vitamins infused as per Ørskov* et al. (1979). *Standard error of means for N retention was* 0·016

Period no.	1	2	3	4	5	6
Duration (d)	4	8	5	5	5	5
Infusion:						
Ac:Pr:Bu (mmol/mol)	650:250:100	860:0:140	860:0:140	860:0:140	860:0:140	860:0:140
Glucose (g)	0	0	26	38	52	0
N retention (mg N/kg $W^{0.75}$ per d)	0	−0·12	−0·02	0·04	0·07	−0·06

N retention responded to propionate and glucose supply reflecting competition for amino acids between protein synthesis and gluconeogenesis. Assuming 1 g protein = 0·58 g glucose, P2 indicates a glucose requirement of at least 2·44 g/kg $W^{0.75}$ per d, no allowance being made for gluconeogenesis from endogenous lactate and glycerol. Comparisons for P1 *v.* P5 and P2 *v.* P6 suggest that net efficiency of amino acid utilization for protein synthesis is increased by a period of 'exogenous glucose' deprivation. This may reflect improved metabolic husbandry of amino acids for protein synthesis or increased glucose sparing and a reduced gluconeogenic demand for amino acids. The latter is consistent with blood plasma glucose, 3-hydroxybutyrate and free fatty acid concentrations.

We are grateful to Dr E. R. Ørskov for his help and advice and to Mr J. McCann for skilled technical assistance.

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The role of attitudes and nutritional knowledge in fat consumption. By R. SHEPHERD and L. STOCKLEY, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA*

The relation between attitudes and food choice is unclear (Foley *et al.* 1979), possibly due to the diversity of definitions of attitudes. We have used the attitudes model of Ajzen & Fishbein (1980), commonly used in social psychology, in which attitudes are assessed in relation to behaviour (e.g. eating certain foods). Subjects (210) completed a questionnaire. This included a nutritional-knowledge component, along with questions on four types of foods contributing highly to fat in the diet (meat, meat products, butter/margarine and milk). For each food there was a question on the frequency of consumption (behavioural intention), and two questions designed to assess the attitude to the behaviour. These involved rating eating of the food on scales labelled unpleasant/pleasant and harmful/beneficial. The other component of this model is the subjective norm, which relates to how the individual thinks other people think he or she should behave. This was assessed with one question for each food.

	Attitude to behaviour		Subjective norm			
Food	Simple r	W_1	Simple r	W_2	Multiple R	No. of subjects
Meat	0·62	0·51	0·46	0·22	0·65	201
Meat products	0·58	0·53	0·32	0·12	0·59	198
Butter/margarine	0·45	0·39	0·31	0·18	0·48	200
Milk	0·42	0·30	0·39	0·20	0·45	193
Total	0·68	0·59	0·49	0·18	0·69	183

All simple and multiple correlation coefficients were significant at $P<0·001$.

The results of multiple regressions, and simple correlations, between the attitude to the behaviour and subjective norm against behavioural intention are shown in the Table for each food and for the total of the four foods. The relative importance of the two components in the multiple regressions are given by the weightings (W_1 and W_2), which are the beta (or standardized) coefficients. The nutritional knowledge score did not correlate significantly with the attitude to the behaviour (r 0·01), subjective norm (r 0·04) or behavioural intention (r −0·03).

The Ajzen & Fishbein (1980) approach showed good prediction of the reported consumption of both the individual foods and the total. The person's own attitude proved a better predictor than the subjective norm. The nutritional knowledge score did not relate to attitudes or reported consumption.

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Calculation of fatty acid intake. By A. J. BROADHURST and L. STOCKLEY, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA*

There is considerable current interest in fatty acid consumption, particularly in relation to coronary heart disease. In epidemiological studies it is impractical to analyse duplicate diets (DD) to estimate fatty acid consumption, consequently weighed intake records (WI) and food composition tables (FCT) are often used.

Eleven subjects collected weighed DD over 16 d, in conjunction with WI. Fatty acid intake was calculated from the WI using four approaches: (A) using standard FCT (Paul & Southgate, 1978) supplemented with additional data (Wiles *et al.* 1980; unpublished data from the Dunn Nutrition Unit), (B) as in (A) with fried and roast foods recoded as boiled or poached foods and the appropriate amount of fats, (C) twenty foods for which fatty acid data was not available but which contributed significantly to the total fat consumption of the subjects, were analysed and added to the FCT (manufacturers' information on brand name oils and margarines was also used), (D) a combination of (B) and (C).

Table 1. *Mean intake by analysis and by calculated methods A–D* (n 176)

	Analysed		A		B		C		D	
Fatty acids	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
18:2	11·5	0·50	6·2***	0·25	6·7***	0·26	9·8***	0·46	10·2**	0·46
18:3	1·4	0·70	0·8***	0·03	0·9***	0·03	1·1***	0·05	1·1**	0·05
Total polyunsaturated	13·0	0·52	7·8***	0·28	8·1***	0·31	11·6**	0·51	12·4	0·52
Total monounsaturated	28·5	0·75	24·3***	0·75	24·2***	0·78	26·9*	0·83	28·6	0·87
Total saturated	32·7	0·82	31·3	0·95	30·8*	0·96	32·7	0·98	34·5**	1·01
Polyunsaturated/ saturated	0·4	0·02	0·2***	0·01	0·2***	0·01	0·3**	0·01	0·3**	0·01

Significantly different from analysed results: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

Table 2. *Correlation coefficients between each calculated method, A–D, and analysed results* (*df* 174)

Fatty acids	A	B	C	D
18:2	0·358	0·384	0·623	0·653
18:3	0·144 NS	0·132 NS	0·123 NS	0·132 NS
Total polyunsaturated	0·375	0·388	0·609	0·638
Total monounsaturated	0·536	0·588	0·560	0·635
Total saturated	0·680	0·686	0·682	0·744
Polyunsaturated/saturated	0·289	0·294	0·464	0·564

All values significant at $P<0·001$, unless otherwise indicated.
NS, not significant.

With methods C and D the correlations generally showed marked improvements and mean values closer to the analysed values. In conclusion, if FCT are to be used for the assessment of fatty acid consumption, consideration should be given to supplementing FCT with fatty acid data on high-fat foods on which UK data has not been published, and recoding fried and roast foods.

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The effect of supplementary phosphate in the diet of lactating cows on the salt composition and heat stability of milk. By J. L. CLAPPERTON, C. HOLT and A. W. M. SWEETSUR, *Hannah Research Institute, Ayr KA6 5HL*

The concentrations of milk-salts, the milk pH and the partition of salts between casein micelles and serum are factors thought to be important in determining the heat stability of milk and milk products. An attempt was made to manipulate milk-salt composition by supplementing a basal diet of hay and concentrates with a mixture of sodium dihydrogen and disodium hydrogen phosphates. Two cows were offered the basal diet in 10 consecutive weeks and in weeks 4, 7 and 8 supplementary phosphate amounting to 116 g P/d was given also. Samples of blood and milk were collected and the averaged results of analysis are shown in Table 1.

Table 1. *Concentrations (mM) of calcium, magnesium and inorganic phosphate (P_i)*

	Blood			Skim-milk				
	Total			Total			Ultrafiltrate	
	Ca	Mg	P_i	Ca	P_i	pH	Ca	P_i
Basal diet	2·53	0·80	1·85	31·8	24·8	6·62	8·6	14·4
Supplement	2·50	0·70	2·53	31·7	23·9	6·63	8·7	13·8
SED	0·098	0·070	0·205	0·18	0·31	0·005	0·10	0·28

Thus, although the dietary supplement of P_i reduced blood Mg and raised blood P_i, there were no significant changes in the concentrations or partitioning of the milk-salts studied. There were, however, differences in heat stability as shown in Table 2, although these cannot be related to milk-salt composition.

Table 2. *Coagulation times (min) at the pH maximum of skim-milk heated at 140° and concentrated skim-milks of 22·5% total solids heated at 120°*

	Basal diet	Supplemented diet	SED
Skim-milk	18·1	14·5	1·1
Concentrate	11·3	12·3	0·9
Pre-heated concentrate	18·4	24·1	3·7

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Is it time to change the standard lactose tolerance test? By K. TADESSE and BETTY WONG, *Department of Physiology, Chinese University of Hong Kong, Shatin, NT, Hong Kong*

Lactose malabsorption is a common condition in the great majority of the adult world population (Ransome-Kuti, 1977). The loss of lactase activity in most persons is partial and intolerance depends on the amount of lactose consumed at a time (Newcomer & McGill, 1984). For clinical practice, the standard lactose tolerance test which employs a dose of lactose equivalent to that found in 1 litre milk (i.e. 50 g) is inappropriate as it does not take account of the individual variation in tolerance. This may lead to falsely attributing symptoms caused by other gastrointestinal disorders to lack of intestinal lactase activity. It would be preferable if the test was designed to determine the degree of lactose intolerance. With the breath hydrogen test this may be possible. To examine the feasibility of such a test, we conducted a study in a group of malabsorbers to determine the amount of lactose they can tolerate.

Twelve adult Hong Kong Chinese (eight male, four female), aged between 20 and 45 years, were given graded volumes of pasteurized cow's milk in a random order on separate days after fasting for 12 h. Malabsorption was assessed by measuring the concentration of H_2 in end-expiratory breath samples. H_2 is a colonic metabolic by-product of anaerobic bacterial fermentation of the unabsorbed sugar and most of it is absorbed into the circulation and excreted through the lungs (Tadesse *et al.* 1980). A sustained rise to 1·0 μmol/l (≃ 20 ppm) or above, 1–3 h after drinking the milk was taken as a positive sign for malabsorption. The results are summarized in the Table:

Level of first sign of malabsorption to lactose of twelve subjects

Amount of milk (ml)	50	100	150	200	250	300	350
Amount of lactose (g)	2·5	5·0	7·5	10·0	12·5	15·0	17·5
Number of subjects with signs of malabsorption	0	2	2	1	2	3	2

The results in the Table indicate that each individual has a specific level of tolerance. This level, though not precise, could be established through a series of breath H_2 tests using graded volumes of milk. Such a test, besides being simple, realistic and acceptable to the patient will provide results which facilitate diagnosis and allow rational dietary advice and management.

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Faecal bile acid profiles and colonic carcinogenesis. By M. S. SIAN, C. BACKHOUSE and T. COOKE, *Department of Surgery, Charing Cross and Westminster Medical School, London W6 8RF*

Faecal bile acids have been implicated in the aetiology of colonic carcinogenesis on the basis of increased concentration in the faeces of high-risk populations (Hill, 1983). The concentration of bile acids in the faeces is influenced by diet and the secondary bile acids, deoxycholic and lithocholic acids, are suggested to be important in colon carcinogenesis (Reddy, 1975). The concentration of the secondary bile acids and the faecal bile acid profile is also affected by the colonic bacteria and bowel function. In the present study, we have examined the bile acid profiles in the faeces of patients with colonic cancer and in healthy Caucasian controls without colonic disease.

A 3-d stool sample was collected by all subjects and faecal bile acids analysed by gas chromatography (Sian, 1982). Total bile acid excretion (see Table) and the excretion of individual bile acids, lithocholic, deoxycholic, chenodeoxycholic and cholic acids, were estimated. The ratio, primary:secondary bile acids, and the proportions of individual bile acids were calculated.

Bile acid excretion (mg/g dry weight of faeces) for ten subjects per group

	Mono-hydroxy		Di-hydroxy		Tri-hydroxy		Total bile acids	
Subjects	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Controls	3·09	0·74	6·94	1·64	0·17	0·11	10·02	2·21
Colon cancer	3·50	1·04	5·19	1·91	0·56	0·19	8·89	1·86

Comparison of faecal bile acid profiles showed marked differences in the excretion of individual bile acids. The largest changes were seen in the proportions of lithocholic, iso-lithocholic and deoxycholic acids. Of these, lithocholic and deoxycholic acids have been reported to show a strong association and to act as promotors of colonic cancer in animal studies.

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