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## **PROCEEDINGS OF THE NUTRITION SOCIETY**

### **ABSTRACTS OF COMMUNICATIONS**

*The Three Hundred and Ninety-seventh Meeting of the Nutrition Society was held in the Chapman Art Gallery and Chapman Lecture Theatre, University of Salford, on Tuesday, 24 April 1984, when the following papers in the form of posters were presented:*

**Effect of diet composition on digestive development of early-weaned pigs.**

By K. J. McCracken, *Agricultural and Food Chemistry Research Division, Department of Agriculture and The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*

Apart from the recent communication by Hampson & Kidder (1984), relatively little information is available as to the changes in digestive development of piglets in the immediate post-weaning period and there is a dearth of information on the effects of diet composition on carbohydrate digestion.

The results reported here relate to three typical commercial diets given to pigs weaned at 14 d. Diets for treatments T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub> contained respectively (g/kg): 413, 255 or 0 dried skim milk and 0, 250 or 320 uncooked cereals. Pigs (eight litters) were sow-reared to 14 or 21 d (T<sub>1</sub>, T<sub>2</sub>) or given the appropriate pelleted diet to appetite for 7 d (T<sub>3</sub>–T<sub>5</sub>). Samples of gut mucosa were collected from anaesthetized pigs at five equally spaced sites of the small intestine (SI) for the determination of the activity of three carbohydrases. The pigs were immediately killed and several organs were removed and weighed.

Treatment . . .	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	Significance	SED
Carcass wt (kg)	4.0	6.0	5.0	5.1	4.5	***	0.37
SI wt (g)	151	209	207	222	209	**	23.2
Pancreas wt (g)	5.6	9.0	11.0	12.0	10.2	***	1.16
SI mucosa wt (g)	65	72	57	49	55	NS	13.4
Protein (mg/g mucosa)	119	101	84	87	84	***	7.4
Enzyme activity (μmol/min per g mucosa)							
Lactase							
(EC 3.2.1.23)	27.7	18.1	11.4	10.0	9.4	***	2.25
Maltase 2†	4.0	6.0	4.4	9.4	7.6	***	1.34
Maltase 3†	0.5	1.3	2.2	4.8	4.1	***	0.69

NS, not significant.

Significance of difference (analysis of variance): \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

† Amyloglucosidase (EC 3.2.1.3).

The results indicate post-weaning reductions in weight of SI mucosa, protein content of mucosa and lactase activity irrespective of diet composition. However, the marked increases in maltase 2 and maltase 3 of T<sub>4</sub>, T<sub>5</sub> pigs compared with sow-reared and T<sub>3</sub> pigs suggest that the nature of the dietary carbohydrate can alter the extent of enzyme induction in the immediate post-weaning period. Within treatments, enzyme activity tended to be positively correlated with food intake. Further studies are required to determine whether food intake was being limited by digestive enzyme activity or whether enzyme induction was secondary to food intake, but it would appear that post-weaning food intake is limited by factors other than digestive enzyme activity.

Hampson, D. J. & Kidder, D. E. (1984). *Proceedings of the Nutrition Society* 43, 18A.

**Effect of diet and post-weaning food intake on digestive development of early-weaned pigs.** By K. J. McCracken and DENISE KELLY, *Department of Agricultural and Food Chemistry, The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX*

McCracken (1984) observed that there was some correlation between food intake post-weaning and digestive enzyme induction and it appeared that the primary factor in post-weaning inappetence was not a lack of digestive enzyme capacity. In order to test this hypothesis it was decided to control post-weaning food intake and examine the changes in digestive development. Pigs (six litters) were weaned at 14 d and slaughtered (T<sub>1</sub>) or fed by gastric intubation six times daily for 3 d prior to slaughter (T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>). T<sub>2</sub> pigs were given 100, 150, 200 g/d of a commercial milk-replacer. T<sub>3</sub> and T<sub>4</sub> pigs received a weaner diet containing 150 g dried skim milk/kg in similar quantities to T<sub>2</sub> pigs (T<sub>3</sub>) or restricted to 0, 50, 60 g/d (T<sub>4</sub>) to represent typical intakes of pigs given dry diets to appetite. Slaughter procedures and measurements were similar to those described by McCracken (1984).

Treatment . . .	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	Significance	SED
SI wt (g)	173	183	175	138	••	11.1
SI mucosa wt (g)	85	93	79	48	••	10.9
Protein (mg/g mucosa)	120	86	79	77	•••	8.6
Enzyme activity (mmol/min per g mucosa)						
Lactase						
(EC 3.2.1.23)	1.16	0.69	0.44	0.33	•	0.22
Maltase 3†	0.08	0.16	0.18	0.09	•	0.028

Significance of difference (analysis of variance): • $P < 0.05$ , •• $P < 0.01$ , ••• $P < 0.001$ .

†Amyloglucosidase (EC 3.2.1.3).

The results confirm the rapid post-weaning induction of amyloglucosidase activity with cereal-based diets. The marked reduction in weights of small intestine (SI) and SI mucosa in T<sub>4</sub> pigs and the reduction in lactase activity in weaned pigs suggest that post-weaning villous atrophy may be more related to the lack of a continuous supply of substrate than to the antigenicity of the diet (Hampson *et al.* 1984).

Hampson, D. J., Kidder, D. E. & Hampson, E. M. (1984). *Proceedings of the Nutrition Society* **43**, 19A.

McCracken, K. J. (1984). *Proceedings of the Nutrition Society* **43**, 109A.

**Further studies of gut enlargement in artificially reared rat pups.** By J. TONKISS and J. L. SMART, *Department of Child Health, The Medical School, Oxford Road, Manchester M13 9PT* and J. EDMOND and N. S. AUESTAD, *Department of Biological Chemistry, UCLA School of Medicine, USA*

It has been demonstrated previously that rats artificially reared (AR) from 5 postnatal days of age show enlargement of certain parts of their gastrointestinal (GI) tracts by 20 d, and that these effects depend to some extent on the type of milk substitute given (Smart *et al.* 1983). The development of these effects was investigated in the present study.

Rat pups were reared normally or were AR from 5 d on one of two milk substitutes. These were the Messer formula, high in carbohydrate and low in protein compared with rats' milk (Messer *et al.* 1969), and the Auestad formula which resembles the composition of rats' milk more closely (Smart *et al.* 1983). Pups AR on these formulae are termed ARM and ARA respectively. ARM and ARA pups received identical amounts of milk substitute daily (2 ml/d on day 5, rising to 8 ml/d by day 20). These amounts were probably less than those taken by MR pups. A total of thirty mother reared (MR), twenty-three ARM and nineteen ARA pups were autopsied at 7, 12 and 20 d. Stomach wet weight and small intestine (SI) length were recorded as previously, and also caecum wet weight and large intestine (LI) length.

	Age (d)	Body-wt	Stomach-wt	SI length	Caecum-wt	LI length
ARA as % MR	7	87*	106	89*	76***	99
	12	70***	186***	107†	119*	94
	20	84***	115**	108*	124**	99
ARM as % MR	7	87**	90	103	87*	99
	12	72***	104	122**	123†	100
	20	86***	98	128***	153***	101
ARA as % ARM	7	99	117*	86*	87	100
	12	97	179***	88*	97	94
	20	98	117**	84***	81	98

† $P < 0.1$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared to reference group (MR in first two comparisons, ARM in the last).

Consistent with our earlier findings for 20 d AR rats (Smart *et al.* 1983), both AR groups had longer SIs than MR rats, the effect being more pronounced in the ARM group, and ARA rats had the heaviest stomachs. Caecum weight, like SI length, was greater in both AR groups than in MR rats. All of these effects were already evident at 12 d. There were even some differences only 2 d after the start of AR, although these were all in the direction of deficits in the size of parts of the GI tract. The remarkable enhancement of GI tract growth occurred after 7 d of age.

Messer M., Thoman, E. B., Terrasa, A. G. & Dallman, P. R. (1969). *Journal of Nutrition* **98**, 404-410.

Smart, J. L., Tonkiss, J., Stephens, D. N., Edmond, J. & Auestad, N. S. (1983). *Proceedings of the Nutrition Society* **42**, 154A.

**Lingual lipase in neonatal fat digestion.** By L. J. SMITH, S. KAMINSKY and S. W. D'SOUZA, *Department of Child Health, St Mary's Hospital, Hathersage Road, Manchester M13 0JH* (Introduced by J. L. SMART)

Lingual lipase (LL; EC 3.1.1.3) secreted by von Ebner's glands at the back of the tongue has been shown to hydrolyse fat in the stomach (Hamosh *et al.* 1981). Indeed, it would appear that newborn infants depend to a greater extent than older infants on adequate hydrolysis of milk triglycerides in the stomach because of lower levels of pancreatic lipase and bile salt concentration in the duodenum (Zoppi *et al.* 1972).

We have studied LL activity in two groups of ten preterm infants (gestational age 26–36 weeks) who were tube-fed. In the first group, gastric aspirates were collected before milk feeds were started and in the second group, after milk feeds. In six infants gastric aspirates were collected before starting milk feeds and on days 1–4 of milk feeding. The study was extended to investigate the extent of fat hydrolysis, together with LL activity, and pH in gastric aspirates from three groups of babies on 1 hourly ( $n$  5), 2 hourly ( $n$  4) and 3 hourly ( $n$  4) feeding regimens. All the infants were fed on formula milk. LL activity in gastric aspirates was determined by [ $^3$ H]triolein hydrolysis *in vitro* (Bläckberg *et al.* 1977); results are expressed as  $\mu\text{mol}$  free fatty acids released/ml of aspirate per h. The products of triglyceride hydrolysis were determined by densitometry.

We observed a significant rise in mean LL activity after starting feeds (mean (SE) enzyme activity before starting feeds was 1.59 (0.70) *v* 4.68 (0.86)  $\mu\text{mol}/\text{ml}$  per h after starting feeds;  $t$  2.79,  $P < 0.02$ ). In the first 4 d after starting milk feeds, LL activity ranged from 0.16 to 9.30  $\mu\text{mol}/\text{ml}$  per h. There was much day-to-day variation in enzyme activity and no systematic pattern. In the three feeding regimens studied the main products of triglyceride hydrolysis were diglycerides and free fatty acids. Mean (SE) triglyceride hydrolysis was 55 (9) % complete after 1 h, 60 (5) % complete after 2 h and 57 (4) % after 3 h. In the 3-hourly fed infants the mean gastric pH fell from 5.52 (range 4.73–6.16) to 1.94 (range 1.43–2.47) over 3 h and mean LL activity rose from 1.89 (range 0.032–6.3) 15 min after a feed to a maximum of 18.97 (range 17.8–19.6) 2 h after feeding.

In prematurely-born infants, LL activity increases after milk feeds and it is suggested that the triglyceride hydrolysis which takes place in the stomach contributes significantly to the nutrition of these infants.

Bläckberg, L., Hernell, O., Fredrikzon, B. & Akerblom, A. K. (1977). *Acta Paediatrica Scandinavica* **66**, 473–477.

Hamosh, M., Scanlon, J. W., Ganot, D., Likel, M., Scanlon, K. B. & Hamosh, P. (1981). *Journal of Clinical Investigation* **67**, 838–846.

Zoppi, G., Andreotti, G., Pajno-Ferrara, F., Njai, D. M. & Gaburro, D. (1972). *Pediatric Research* **6**, 880–886.

**Mucosal morphology in the alimentary tract of calves given heated soya-bean flour.** By J. W. SISSONS, H. E. PEDERSEN and KAREN WELLS, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Previous studies of preruminant calves given feeds containing heated soya-bean flour (HSF) showed that sensitization and challenge-induced abnormalities in digesta movement and nutrient absorption, increased gut permeability to protein macromolecules and provoked high titres of circulatory IgG antibodies specifically against soya-bean globulins (Sissons & Smith, 1976; Kilshaw & Sissons, 1979; Kilshaw & Slade, 1980). Further work reported here has examined changes in mucosal architecture in response to HSF feeds.

Four Friesian bull calves equipped with abomasal and ileal re-entrant cannulas received a series of test feeds containing HSF as the protein source at intervals of 2–3 d from about 6 weeks of age. Diets, feeding procedures (by abomasal infusion) and digesta collection at the distal ileum have been described by Sissons & Smith (1976). When measurements of digesta flow indicated the development of digestive disorders, calves were given further feeds of HSF (calves A, B and C) or casein (calf D) and about 5 h later they were anaesthetized with a halothane–oxygen mixture. Intestinal segments were fixed *in situ* using cacodylate-buffered glutaraldehyde (30 g glutaraldehyde/l, pH 7.2), removed and then prepared by the 'OTOTO' method (Malick *et al.* 1975) for scanning electron microscopy (SEM). Abomasal tissue was also sampled, fixed and prepared for SEM. Other tissue samples were similarly obtained from an intact milk-fed animal (calf E).

Mucosal architecture of tissue samples from calf D was similar to that of calf E. Abomasal and proximal duodenal surfaces appeared ridged and convulated with no villi, whereas in the distal duodenal, jejunal and ileal material, numerous villi were seen as compact, slender, finger- or tongue-like structures with transverse furrows and the openings of goblet cells on otherwise smooth surfaces. In contrast, micrographs of abomasal and duodenal tissue from calves A, B and C showed ridges surrounding the openings of crypts but with the epithelium in between appearing grossly disorganized. Irregularities were also observed in jejunal and ileal tissue; villi were short and fat with extremely abnormal extrusion zones. Thus, villus tips were damaged with signs of epithelial cell extrusion and the emergence of red and white blood cells. Most villi were surrounded by a matrix of fibrin strands entrapping blood cells, bacteria and particles of undigested dietary protein. Digesta samples of these calves contained large quantities of fibrin in the form of tubular casts.

It is concluded that in calves given HSF, abnormal digesta movement and impaired nutrient absorption is related to morphological disturbances in mucosal structure arising from an allergic reaction in the gastrointestinal tract.

H.E.P. acknowledges receipt of financial support from Aarhus Oliefabrik A/S and the Royal Veterinary and Agricultural University, Denmark.

- Kilshaw, P. J. & Sissons, J. W. (1979). *Research in Veterinary Science* 27, 366–371.  
Kilshaw, P. J. & Slade, H. (1980). *Clinical Experimental Immunology* 41, 575–582.  
Malick, L. E., Wilson, R. B. & Stetson, D. (1975). *Stain Technology* 50, 265–269.  
Sissons, J. W. & Smith, R. H. (1976). *British Journal of Nutrition* 36, 421–437.

**Histologic and metabolic responses to soya-bean antigens in the gut of the preruminant calf.** By H. E. PEDERSEN, J. W. SISSONS and A. TURVEY, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT* and I. SØNDERGAARD, *Laboratory for Medical Allergology, Rigshospitalet, Copenhagen, Denmark*

Previously (Sissons *et al.* 1984) we reported morphological disturbances in the mucosal structure of calves given heated soya-bean flour (HSF) which contained antigenically-active proteins glycinin and  $\beta$ -conglycinin. We also made a quantitative assessment of mucosal damage in different regions of the small intestine in four calves sensitized to HSF and anaesthetized after a further feed of either HSF (calves A, B and C) or casein (calf D). Samples of mucosal tissue were removed and examined according to the microdissection technique of Ferguson *et al.* (1977). Other segments were fixed *in situ*, removed, stained with toluidine blue and examined under a light microscope. Tissue samples were also taken from three intact milk-fed animals (calves E, F and G).

Compared with intestinal tissue from calves D, E, F and G, specimens from HSF-fed animals (A, B and C) showed notable reductions in villous heights and some elongations of crypts particularly in the duodenum and mid-jejunum (see Table). Shortening of villi was also accompanied by an increase in width. Histological examinations also revealed a higher rate of mitosis in crypt cells and increased numbers of mast cells in the lamina propria of tissue from calves A, B and C.

	Distance from pylorus (m)		Villous height ( $\mu\text{m}$ )						Crypt depth ( $\mu\text{m}$ )							
	(n 7)		Feed...		Casein		Milk (n 3)		HSF (n 3)		Casein		Milk (n 3)		HSF (n 3)	
	Mean	SE			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Duodenum	0.10	0.01			247	359	68	214	28	493	340	44	591**	34		
Proximal jejunum	3.14	0.44			853	845	98	492*	39	424	416	88	459	43		
Mid-jejunum	9.56	0.18			875	993	177	476*	12	432	393	37	488	20		
Ileum	17.94	0.75			408	440	14	452	53	456	370	27	476*	15		

Individual values were means of thirty-forty measurements.

Significance of difference (analysis of variance): \* $P < 0.05$ , \*\* $P < 0.01$ .

To study the possible involvement of histamine release, urine collected before and after calves received HSF or casein feeds was analysed for a known metabolite 1,4-methyl-imidazolacetic acid (Søndergaard, 1982). However, there was no change in excretion of this substance after challenge with HSF-feeds.

Our results indicate that villous heights vary in a regular manner and are longest in the mid-gut. This region appears to be most sensitive in exhibiting villous atrophy in calves given antigenic soya-bean flour.

Ferguson, A., Sutherland, A., MacDonald, T. T. & Allan, F. (1977). *Journal of Clinical Pathology* **30**, 1068-1073.

Sissons, J. W., Pedersen, H. E. & Wells, K. (1984). *Proceedings of the Nutrition Society* **43**, 113.

Søndergaard, I. (1982). *Allergy* **37**, 581-586.

**Soya-bean antigen survival in the digestive tract of the preruminant calf.**

By J. W. SISSONS, H. E. PEDERSEN and SHEILA M. THURSTON, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Earlier studies have shown that intolerance of heated soya-bean flour (HSF) by preruminant calves is related to the presence of two antigenically-active globular proteins, glycinin and  $\beta$ -conglycinin, in the soya-bean product (Kilshaw & Sissons, 1979). The present work examined the possibility that these antigens survive digestion in the calf alimentary tract.

Haemagglutination inhibition assay (HIA) described by Kilshaw & Sissons (1979) was used to measure soluble glycinin and  $\beta$ -conglycinin in abomasal and intestinal digesta samples obtained from three cannulated calves given feeds containing HSF. When measurements of digesta passage indicated provocation of a gastrointestinal hypersensitive response (Sissons & Smith, 1976), animals were anaesthetized and digesta sampled from the tract. Soluble antigens were also assayed in digests prepared by incubating HSF in vitro with and without different proteases (standard rennet containing mainly rennin (*EC* 3.4.24.4), stabo rennet containing mainly pepsin (*EC* 3.4.23.1) and purified bovine trypsin (*EC* 3.4.21.4)) at optimal pH conditions of pH 3.5, 1.8 and 7.8 for rennin, pepsin and trypsin respectively.

HIA of diet samples and digesta collected about 6 h after a HSF feed gave mean titres ( $\log_2$ ) for glycinin of 13, 7, 9, 10 and 9 and  $\beta$ -conglycinin of 11, 8, 10, 8 and 8 for diet, abomassal, duodenal, jejunal and ileal samples respectively (results for three animals). HIA titres of HSF in vitro digests (see table) revealed that  $\beta$ -conglycinin, but not glycinin, was unaffected by pepsin and that both antigens were fairly resistant to rennin and trypsin. Measurements of digests without enzymes showed that the solubility of glycinin and  $\beta$ -conglycinin remained high over pH ranges likely to be encountered in the calf digestive tract.

Digesta	Highest dilution ( $\log_2$ ) inhibiting agglutination by:	
	Anti-glycinin	Anti- $\beta$ -conglycinin
Standard rennet	12	11
No enzyme, pH 3.5	12	9
Stabo rennet	neg	12
No enzyme, pH 1.8	9	12
Bovine trypsin	12	7
No enzyme, pH 7.8	12	12

neg = Negative result at dilution 1:10.

Titres for standard dilutions of glycinin (46  $\mu\text{g/ml}$ ) and  $\beta$ -conglycinin (47  $\mu\text{g/ml}$ ) were 6 and 7 respectively.

Results presented here suggest that calves suffer from gastrointestinal allergic reactions to HSF because, apart from the action of pepsin on glycinin under optimal conditions, proteases of the digestive tract do not readily denature soluble antigenic constituents of globular soya-bean protein.

Kilshaw, P. J. & Sissons, J. W. (1979). *Research in Veterinary Science* 27, 366-371.  
Sissons, J. W. & Smith, R. H. (1976). *British Journal of Nutrition* 36, 421-437.

**Immune hypersensitivity and post-weaning diarrhoea in the pig.** By B. G.MILLER<sup>1</sup>, A. D. PHILLIPS<sup>2</sup>, T. J. NEWBY<sup>1</sup>, C. R. STOKES<sup>1</sup> and F. J. BOURNE<sup>1</sup>,<sup>1</sup>*Department of Veterinary Medicine, University of Bristol, Langford House, Langford, Bristol BS18 7DU* and <sup>2</sup>*Queen Elizabeth Hospital for Children, Hackney Road, London E2 8PS*

Stokes *et al.* (1984) reported that the presentation of a novel antigen in mice induces a cell-mediated immune hypersensitivity prior to the development of immune tolerance. This is associated with increased crypt cell mitosis and malabsorption. Miller *et al.* (1984) have postulated that such a transient immune hypersensitivity underlies the aetiology of post-weaning diarrhoea in the pig.

Unweaned pigs show antigen-specific changes in gut function and morphology following the presentation of ovalbumin (2 g/d) that are qualitatively similar to those reported in mice by Stokes *et al.* (1984). To evaluate further this hypothesis, three experimental groups of pigs were weaned at 3 weeks of age on to a diet in which the only source of protein (220 g/kg dry diet) was full fat soya-bean flour. In Expt 1, groups of pigs ( $n$  5–6) were killed at 0, 1, 2, 5, 7 and 13 d post-weaning. 24 h prior to slaughter, each pig was injected intracutaneously in one ear with 0.1 ml of a sterile solution of the full fat soya-bean flour (<1 mg/ml). At slaughter, tissue samples were taken from the site of injection in the ear and from 25% and 75% along the length of the small intestine. Inflammation of the ear was assessed by measuring any increase in ear thickness with an engineers' micrometer. In Expt 2, xylose absorption was measured at 0, 2, 5 and 11 d post-weaning by orally dosing pigs ( $n$  8; however, two pigs died from an *Escherichia coli* enteritis during the experimental period) with a solution of D-xylose (100 g/l; 1 ml/kg body-weight) and measuring serum xylose concentration 1 h later. The pigs ( $n$  6) in Expt 3 were fed, prior to weaning, on the full fat soya-bean flour as a supplement to the sow's milk under conditions which maximized intake of the flour. Xylose absorption was then measured 0 and 5 d post-weaning.

Days post-weaning . . .	0		2		5		11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma xylose (mmol/l)	1.07	0.19	0.84	0.44	0.20 <sup>a</sup>	0.12	1.22	0.25
Change in ear thickness ( $\mu$ m)	12	272	490	560	1350 <sup>b</sup>	770	-170	299

<sup>a</sup>Significantly different from unweaned value (paired  $t$  test):  $P < 0.001$ .

<sup>b</sup>Significantly different from value before challenge (paired  $t$  test):  $P < 0.02$ .

The results concurred with the hypothesis that following weaning, pigs experience a transient immune hypersensitivity to dietary antigens. This is associated with a period of malabsorption, diarrhoea, crypt cell hyperplasia, villous atrophy and an increased susceptibility to *E. coli* enteritis. Prior feeding of antigen to induce immune tolerance can prevent both the malabsorption and the diarrhoea.

Miller, B. G., Newby, T. J., Stokes, C. R. & Bourne, F. J. (1984). *Research in Veterinary Science* 35 (In the Press).

Stokes, C. R., Newby, T. J., Miller, B. G. & Bourne, F. J. (1984). *Proceedings of the Nutrition Society* 43, 117A.

**The induction of gut damage as a result of a transient immune hypersensitivity to dietary antigens.** By C. R. STOKES<sup>1</sup>, T. J. NEWBY<sup>2</sup>, B. G. MILLER<sup>1</sup> and F. J. BOURNE<sup>1</sup>, *Departments of <sup>1</sup>Veterinary Medicine and <sup>2</sup>Animal Husbandry, University of Bristol, Langford House, Langford, Bristol BS18 7DU*

The immune system of the gastrointestinal tract is presented with an almost unique dilemma, for whilst a response appropriate to nonreplicating dietary or environmental antigens would provide inadequate protection against potential pathogens, a response that is protective against enteric bacteria and viruses would at best be wasteful or worse have the potential for damaging hypersensitivity reactions to dietary components. An animal may protect itself from such damaging reactions to dietary components by the development of immune exclusion (which reduces the absorption of a specific antigen) and oral tolerance (which suppresses an animal's ability to respond to antigen that is not excluded). Prior to the induction of this protected state, a transient phase of cell-mediated immunity may be demonstrated (Newby *et al.* 1980).

To determine if similar hypersensitivity reactions occur locally at the site of antigen presentation (i.e. in the gut) at the same time as distally (at skin sites) a group of eight CBA male mice were given daily 200  $\mu$ l sheep red blood cells (SRBC) ( $3 \times 10^9$  cells/d) by gastric intubation. A positive skin test could be elicited upon challenge after feeding for 4 d, whilst those fed for 14 d were no longer sensitive (i.e. orally tolerized). The local effects were assayed by feeding SRBC for 2 d and challenging after 10 d by feeding SRBC on three consecutive days. Their ability to absorb xylose from a 10 mg feed was then measured on the following day, and xylose levels were significantly lower in these mice in comparison with: (1) those challenged but unsensitized; (2) those challenged with SRBC but sensitized with the non-cross-reacting antigen horse red blood cells; and (3) those orally tolerized by feeding SRBC for 14 d. Comparison of the intestines of mice killed at this time showed that the rate of production of crypt cells and the numbers of intraepithelial lymphocytes were higher in those sensitized and challenged with SRBC.

The gut changes induced in mice given SRBC show antigen specificity and memory, confirming that they are immune mediated. Since they occur during the period when animals can elicit delayed type hypersensitivity skin reactions and are histologically similar to those seen during allograft rejection and graft *v.* host reactions (MacDonald & Ferguson, 1976, 1977), it is likely that they are a consequence of a cell-mediated immune reaction to the fed antigen.

MacDonald, T. T. & Ferguson, A. (1976). *Gut* 17, 81-91.

MacDonald, T. T. & Ferguson, A. (1977). *Cell Tissue Kinetics* 10, 301-312.

Newby, T. J., Stokes, C. R. & Bourne, F. J. (1980). *Immunology* 41, 617-621.

**A simple pocket computer program for assessing nutritional requirements.** By C. M. COLLEY and A. FLECK, *Department of Chemical Pathology, Charing Cross Hospital Medical School, London W6 8RF*

The accurate assessment of a patient's nutritional needs is obviously desirable to avoid under- or over-feeding. A program has been written for a Sharp PC-1500 pocket computer, which calculates a 'best estimate' of energy and nitrogen requirements for a patient. The basal metabolic rate is estimated from the patient's body-weight, height, age and sex using the data of Dubois & Dubois (1966) and Robertson & Reid (1952). Modifications are then made to this value to allow for the degree of activity of the patient, the extent of various types of trauma (surgical operation, fractures, burns), sepsis and pyrexia. These are based on data from Kinney *et al.* (1970) and Wilmore (1977).

Preliminary evaluation has shown good correspondence between predicted resting energy requirements and measured resting metabolic expenditure in normal controls and in a variety of patients, with metabolic rates ranging from less than 6.3 MJ/d (1500 kcal/d) to over 12.5 MJ/d (3000 kcal/d), although such high values are found in very few patients, even those with severe sepsis. In no case was a metabolic rate greater than 14.6 MJ/d (3500 kcal/d) measured, although we have not studied any burns patients. In a study of over sixty subjects, measured (*y*) *v.* predicted (*x*) energy expenditure had a gradient of 0.93 (correlation coefficient 0.86). The predicted value allowed for all factors except mobility of the subject, and measurements, using indirect calorimetry, were made after a 1 h rest. N requirement was estimated in relation to the energy requirement, with the value varying from 1 g N/837 kJ (1 g N/200 kcal) in reasonably well patients to 1 g N/418 kJ (1 g N/100 kcal) in severely septic patients. Again, a good correlation was found between measured (*y*) and predicted (*x*) values; the gradient was 0.97 with a correlation coefficient of 0.96.

The program also makes an estimate of sodium, potassium and water requirements based on the energy requirement plus measured fluid losses. The program is written in Basic, and may easily be modified to cater for special circumstances. The computer can be used alone, the numbers being read from the liquid-crystal display, or attached to its portable printer, thus allowing a permanent record of input and output data to be produced.

Dubois, D. & Dubois, E. F. (1966) *Archives of International Medicine* 17, 863-871.

Robertson, J. D. & Reid, D. D. (1952). *Lancet* i, 940-943.

Kinney, J. M., Duke, J. H., Long, C. L. & Gump, F. E. (1970). *Journal of Clinical Pathology* 23, Supplement 4, 65-72.

Wilmore, D. W. (1977). *The Metabolic Management of the Critically Ill*. London: Plenum Medical.

**Nutritional audit—experience of a surgical nutritional advisory group.**

By J. E. A. SQUIRES, A. J. W. SIM, O. J. GARDEN, A. McLELLAND, K. CARR, C. WINTON and A. SHENKIN, *Glasgow Royal Infirmary, Glasgow G4 0SF*

The formation of nutrition teams has been advocated as a means of improving the provision of nutritional care (Harper *et al.* 1983). A multidisciplinary Nutritional Group involving surgeons, biochemists, dietitians, pharmacists and nursing staff was set up in January 1979 to deal with nutritional problems in surgical units in Glasgow Royal Infirmary. Details of clinical conditions, nutritional support and laboratory data were documented on specially designed computer forms. Analysis of the data from the first 306 patients referred to the group has now been performed.

There were 171 males and 135 females with an average age of 59 years (range 16–86 years). Primary diagnosis in 118 patients was neoplastic disease. 226 patients received nutritional support in the post-operative period, eighty-six of whom had enterocutaneous fistulae. Thirty-five patients were fed in the pre-operative period and forty-five patients received nutritional support but did not undergo surgery. One hundred and twenty-two patients received enteral nutrition, 125 intravenous nutrition and fifty-four a combination of both. Five patients underwent nutritional assessment and received oral supplements alone. The duration of courses of nutritional support were similar for patients fed enterally (median 11.5 d; range 1–147 d) and intravenously (median 12.0 d; range 1–111 d).

Intravenous nutrition was provided by 3-litre bag regimens using standard amounts of nutrients and additives and providing 12.6 MJ/d (8.4 MJ as carbohydrate and 4.2 MJ as fat) and 14 g N. In a 3 year period there were 3013 intravenous feeding days, in 55% of which standard regimens were used, 34% required altered regimens as regards fluid and electrolyte content and 11% did not follow the normal regimen, in terms of energy and amino acid provision. Enteral nutrition was also standardized and provided in most patients by a fine bore nasoenteric tube (average total intake 8.4 MJ/d, 10 g N). There has been little change in the relative use of enteral and parenteral nutrition over the period of the study.

Seventy-eight patients did not survive their hospital admission. Mortality in the cancer group was 30% and in the non-cancer group was 23%. The mortality in patients who received both enteral and intravenous nutrition (30%) was similar to patients who received intravenous nutrition alone (30%), whereas 19% of patients who received enteral nutrition alone died.

This type of nutritional audit can provide useful information in detecting trends in the amount and type of nutritional support provided and in the efficacy of such therapy.

Harper, P. H., Royle, G. T. & Mitchell, A. (1983). *British Medical Journal* **286**, 1323–1327.

**Assessment of patients at commencement of nutritional support—relation to type of feeding and clinical outcome.** By A. SHENKIN, C. WINTON, J. E. A. SQUIRES and O. J. GARDEN, *Department of Biochemistry and Surgery, Glasgow Royal Infirmary, Glasgow G4 0SF* and D. HOLE, *Cancer Surveillance Unit, Ruchill Hospital, Glasgow*

Recent studies have indicated the value of pre-operative nutritional measurements in the prediction of post-operative complications (Simms *et al.* 1982) and we have studied this in our patient population.

Of the first 306 patients referred to our Nutritional Advisory Group, assessment data are available on 293. One hundred and forty of these patients were commenced on enteral feeding and 148 on parenteral feeding on clinical grounds. The initial assessment demonstrated significant differences between these groups (Student's *t* test and Mann-Whitney test).

Initial feeding	Body wt (% ideal)	Mid-arm muscle circumference (% standard)	Triceps skinfold thickness (% standard)	Serum albumin (g/l)	Serum ascorbate (μmol/l)	Vitamin B <sub>1</sub> (% activation red blood cell enzyme)	Vitamin B <sub>12</sub> (% activation red blood cell enzyme)
<b>Enteral</b>							
Mean	83.9	79.5	67.5	30.2	22.6	11.9	26.2
SD	15.6	11.7	31.4	6.1	16.5	10.5	22.2
<b>Parenteral</b>							
Mean	93.0	84.7	79.2	28.6	14.2	18.1	32.9
SD	18.0	11.7	31.5	5.1	6.7	11.3	22.1
<i>P</i> <	0.001	0.001	0.01	0.02	0.001	0.001	0.02

Applying the formula of Simms *et al.* (1982) to 205 patients with complete data, forty-three patients who died whilst in hospital had a poorer prognostic factor (64.8, SD 15.8) than 162 patients discharged from hospital (53.9, SD 20.9; *P*<0.001). The mortality in patients with a prognostic factor of less than 70 was 15.5%, whereas mortality was 33.3% in patients with a prognostic factor greater than 70. Many individual variables were also more abnormal in the non-survivors.

Patient	Triceps skinfold thickness (% standard)	Mid-arm muscle circumference (% standard)	Serum						Vitamin B <sub>1</sub> (% activation red blood cell enzyme)	
			Albumin (g/l)	Transferrin (g/l)	P (mmol/l)	Mg (mmol/l)	Zn (μmol/l)	Cu (μmol/l)		Retinol (μmol/l)
<b>Died in hospital</b>										
Mean	63.0	78.8	27.1	1.6	0.89	0.73	10.6	18.1	1.07	19.1
SD	25.3	12.1	5.3	0.6	0.33	0.12	3.2	5.3	0.66	13.4
<b>Discharged</b>										
Mean	75.9	82.8	30.1	1.9	1.00	0.77	11.9	19.3	1.33	13.6
SD	33.2	11.8	5.6	0.7	0.30	0.13	3.9	5.2	0.85	10.1
<i>P</i> <	0.01	0.05	0.001	0.001	0.01	0.05	0.05	0.05	0.05	0.005

Similar results were found for patients whether fed enterally or parenterally, and whether assessed in the post-operative or pre-operative period.

These results indicate that patients receiving enteral or parenteral feeding had a different type of nutritional depletion. It is also concluded that anthropometric and biochemical measurements other than triceps skinfold thickness and serum proteins may be helpful in identifying patients with a poor prognosis.

Simms, J. M., Smith, J. A. R. & Woods, H. F. (1982). *Clinical Nutrition* 1, 71–79.

**Evaluation of protein synthesis rates after surgery in metastatic malignant disease.** By H. C. WARD<sup>1</sup>, A. W. JOHNSON<sup>1</sup>, D. HALLIDAY<sup>2</sup> and A. J. W. SIM<sup>1</sup>, <sup>1</sup>*Academic Surgical Unit, St Mary's Hospital, London W2* and <sup>2</sup>*Clinical Research Centre, Harrow HA1 3UJ*

Rates of whole body protein synthesis and breakdown can be calculated from nitrogen input and output along with the third independent variable, N turnover. A primed continuous 24 h infusion of [<sup>15</sup>N]glycine (Sim *et al.* 1980) was used to achieve a plateau of enrichment of urinary urea from which N turnover is calculated. Plateau enrichment was determined from a single sample from urine collected during the second 12 h of the infusion period (Sim *et al.* 1984). Urinary N excretion was determined for the whole 24 h period. N turnover measurements were commenced at 18.00 hours on the second post-operative day. Two groups of patients have been studied. Group 1 consisted of nine patients (two male, seven female) with non-malignant disease or malignant disease without metastases. Their mean age was 64 (SEM 3) years and mean weight 61 (SEM 3) kg. Group 2 consisted of eight patients (three male, five female) having malignant disease with overt metastases. Their mean age was 65 (SEM 4) years and mean weight 62 (SEM 8) kg. Six patients in each group underwent colonic resection. They received only dextrose solution (50 g/l) and saline post-operatively. Results are shown in the table.

	Group 1 (n 9)		Group 2 (n 8)	
	Mean	SEM	Mean	SEM
N turnover (mg N/kg per h)	458	34	598	66
Synthesis (g protein/d)	127	11	182*	19
Breakdown (g protein/d)	173	14	223*	24
Nitrogen balance (g N/d)	-7.3	1.1	-6.5	1.7

Mann-Whitney U two-tailed test: \* $P < 0.05$ .

The findings of higher levels of protein metabolism in circumstances where there is no nutritional intake suggests autonomous tumour metabolism unaffected by trauma or starvation.

Under these conditions the tumour obtains its nutrition from endogenous sources such as muscle and fat, depletion of which may prejudice survival after major palliative surgery.

Sim, A. J. W., Wolfe, B. M. & Sugden, B. (1980). *Journal of Parenteral and Enteral Nutrition* **4**, 180-183.

Sim, A. J. W., Ward, H., Johnson, A. W. & Halliday, D. (1984). *Proceedings of the Nutrition Society* **43**, 46A.

**Fat emulsion in 'complete nutritional mixtures' for intravenous feeding— a clinical evaluation.** By A. J. W. SIM, H. C. WARD, A. HUMFRIES and H. A. F. DUDLEY, *Academic Surgical Unit and Department of Pharmacy, St Mary's Hospital, London W2*

Although intravenous feeding with mixtures of amino acids, glucose, electrolytes, trace minerals and fat emulsion have been in clinical use since 1973 (Solassol *et al.* 1973), concern about the stability of such nutritive mixtures has precluded them from regular use in the UK. However, following research into the stability of the fat emulsion 'Intralipid' in complete nutritional mixtures (Sjoberg & Jeppsson, 1984), solutions containing fat emulsions, amino acids, glucose, electrolytes, trace minerals and vitamins have been available since 1982.

From September 1982 to January 1984, ninety-six patients have been fed intravenously with solutions containing fat emulsion. All solutions were infused into a central vein from a 3-litre bag with flow controlled by a silicone membrane proportionating valve. A total of 1033 bags have been made in a laminar air flow cabinet under strict aseptic conditions. The solutions were prepared by adding trace minerals to the amino acid solution, phosphate to the dextrose solution, then combining the two resultant solutions in a 3-litre bag. The water-soluble vitamins were reconstituted and fat-soluble vitamins added, the combined vitamin solution was added to the Intralipid and the resultant fat emulsion and vitamin solution mixed with amino acids, minerals and dextrose solution in the 3-litre bag. All solutions have been infused within 72 h of manufacture and none has had to be discarded because of visible 'destabilization' of the fat emulsion.

The median number of 3-litre bags containing the complete nutritive mixture infused was 5 (range of 1–163) bags/patient.

Patient	Infusion	Albumin (g/l)		AP (U/l)		AST (U/l)		Bilirubin (μmol/l)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Non-septic	Before	340	30	138	23	25	6	19	11
	After	340	30	298	83	52	15	8	2
Septic	Before	310	20	194	41	31	5	21	6
	After	290	60	366	229	58	14	21	6

AP, alkaline phosphatase (EC 3.1.3.1); AST, aspartate transaminase (EC 2.6.1.1).

This technique of intravenous nutrition has found acceptance from nursing staff as it represents a simplification in the technique of intravenous nutrient provision by removing the necessity for separate infusion of fat emulsion. Metabolic disturbances which might be attributable to the infusion of the complete nutritional solutions have not been observed.

Solassol, C., Joyeux, H., Serrou, B., Pujol, H. & Romieu, C. (1973). *Journal de Chirurgie* **105**, 15.

Sjoberg, B. & Jeppsson, R. (1984). *Clinical Nutrition* (In the Press).

**A comparison of the absorption from 'chemically-defined elemental' or 'whole protein' enteral feeds by the human small bowel.** By C. A.

RUSSELL, S. J. EVANS and C. MABBUTT, *General Hospital, Cliftonville, Northampton*

A controlled study was undertaken to compare absorption from two different commercially available enteral feeds in patients with established ileostomies. One feed contained short-chain polypeptides with free amino acids, i.e. 'chemically-defined elemental' powdered enteral feed (Nutranel<sup>®</sup>, Cassenne), the other contained long-chain polypeptides without free amino acids, i.e. 'whole protein' liquid enteral feed (Clinifeed 400<sup>®</sup>, Cassenne).

Ten patients with well functioning ileostomies were orally fed for two separate 7 d periods on Nutranel plus free clear fluids and Clinifeed 400 plus free clear fluids. Each regimen provided 8.4 MJ (2000 kcal) and 12.0–12.8 g nitrogen/d. Daily oral intakes were recorded during each 7 d period and analysed for total fluids, energy, N and electrolytes. Palatability, ease of preparation and individual preferences were recorded. Total daily stoma effluents for each patient were collected on days 4 to 7 and analysed for wet and dry weight, N, sodium and potassium.

No statistically significant changes in anthropometric measurements, biochemical or haematological profiles were observed during either study period. The percentage absorptions of fluids, N, Na and K in each group were compared and found not to be statistically different. Two patients indicated a personal preference for Nutranel while seven preferred Clinifeed 400. The table lists the effluent analysis expressed as group daily means, standard deviations and ranges of results for the Nutranel and Clinifeed 400 groups.

	Nutranel			Clinifeed 400		
	Mean	SD	Range	Mean	SD	Range
Wet wt (g)	490	184	231–818	456	264	183–978
Dry wt (g)	25.44	7.70	12.3–35.9	26.48	4.08	11.1–47.8
N (g)	1.38	0.40	0.7–2.0	1.47	0.67	0.7–2.8
Na (mmol)	53.4	20.6	21–92	51.2	25.6	21–102
K (mmol)	6.2	3.7	2–15	6.7	6.8	2–25

These results indicate no significant difference in the absorption by the small bowel of fluids, N or electrolytes between a 'chemically-defined elemental' feed and a 'whole protein' feed, provided there was no pancreatic or biliary insufficiency. However, personal preference, ease of preparation and financial considerations should encourage the use of the liquid 'whole protein' enteral feed.

**Absorption of sugars in sickle cell disease.** By P. O. ODonkor, F. K. ADZAKU and S. K. ADDAE, *Department of Physiology, University of Ghana Medical School, PO Box 4236, Accra, Ghana*

The nitrogen economy of sickle cell patients (HbSS) is impaired because of poor intestinal absorption of protein products and poor N utilization (Odonkor & Addae, 1982; Odonkor *et al.* 1982). Whether or not the absorption of sugars is also impaired is not clear but there is some evidence that this is probably the case (Okafor & Osamo, 1981).

The intestinal absorptive capacity of sickle patients has therefore been evaluated using the glucose tolerance test and the xylose absorption test. Thirty-nine male, young adult Ghanaians, aged 19–27 years, were investigated (fifteen controls and twenty-four HbSS subjects). The glucose tolerance test was done in the conventional way with 100 g glucose in 250 ml water and the xylose test was carried out using a D-xylose solution of 33.3 mmol/l. Blood xylose concentrations were determined after 1 and 2 h and urine xylose was measured at 2 and 5 h after xylose ingestion.

Like the control subjects the peak blood glucose concentration in the HbSS subjects (6.38 (SE 0.05) mmol/l) occurred at 1 h after glucose ingestion, but the level was significantly lower ( $P < 0.01$ ) than that found in the controls (8.44 (SE 0.44) mmol/l). Blood xylose concentration at 1 h after xylose ingestion was also significantly lower ( $P < 0.01$ ) in the HbSS subjects (0.8 (SE 0.4) mmol/l) compared with the controls (1.9 (SE 1.0) mmol/l). The mean 2-h blood xylose level and the urine xylose at 2 h and 5 h were not significantly different in the two groups.

The lower blood glucose peak and 1-h blood xylose concentration in the HbSS subjects suggest reduced absorption of these sugars. The normal urinary xylose excretion by the HbSS subjects may be due to their well-known supra-normal glomerular filtration rate.

This work was supported by generous donations to the Sickle Cell Research Fund of the Department of Physiology, University of Ghana Medical School.

Odonkor, P. O. & Addae, S. K. (1982). *Proceedings of the Nutrition Society* 41, 125A.

Odonkor, P. O., Yamamoto, S. & Addae, S. K. (1982). *West African Journal of Medicine* 1 (5), 21–25.

Okafor, L. A. & Osamo, N. O. (1981). *West African Journal of Medicine* 1 (1), 9–12.

**Vitamin E and selenium status in Crohns disease.** By M. LOUGH<sup>1</sup>, A. MAIN<sup>2</sup>, R. I. RUSSELL<sup>2</sup> and A. SHENKIN<sup>1</sup>, *Departments of <sup>1</sup>Pathological Biochemistry and <sup>2</sup>Gastroenterology, Glasgow Royal Infirmary, Glasgow G4 0SF*

Pathological conditions of the gastrointestinal tract producing steatorrhoea may produce deficiencies of the fat-soluble vitamins. It is established that vitamin E as well as selenium protect cell membranes against the effects of oxidants in vivo.

The present study included twenty-five patients with established Crohns disease. All the patients had plasma vitamin E, Se, glutathione peroxidase (*EC* 1.11.1.9) (plus red cell glutathione peroxidase), haemoglobin (Hb), reticulocyte count, haptoglobin concentration and stability of red blood cells to hydrogen peroxide measured.

Plasma Se concentration had a mean value (1 SD) of 1.21 (0.46)  $\mu\text{mol/l}$  with only two patients having low values (less than 0.6  $\mu\text{mol/l}$ ). Mean red cell glutathione peroxidase (1 SD) was 17.6 (6.8) U/g Hb (normal range 13–25 U/g Hb), five patients having abnormally low values.

Median plasma vitamin E concentration was 16  $\mu\text{mol/l}$  (range 1–50  $\mu\text{mol/l}$ ), eight patients having low values (less than 12  $\mu\text{mol/l}$ ). The stability of red blood cells to hydrogen peroxide was grossly impaired, the median value being 83% haemolysis, nineteen patients having abnormal values (i.e. greater than 10% haemolysis). There was a significant correlation between vitamin E levels and hydrogen peroxide stability ( $r$  -0.68). All patients with low vitamin E concentrations had abnormal hydrogen peroxide stability.

Twenty-four hour fat excretion was measured in sixteen patients, the median value being 73 mmol/24 h (range 14–179 mmol/24 h). Twelve patients had steatorrhoea (i.e. greater than 21 mmol fat 24 h), and all patients with low plasma vitamin E concentrations were in this group.

The relevance of the above findings to in vivo haemolysis was studied. There was no relation between vitamin E concentration, Se concentration, hydrogen peroxide stability in vitro and fat excretion on the one hand, with reticulocyte count and serum haptoglobin concentration on the other. There was no evidence of significant in vivo haemolysis, since no patient had a reticulocyte count greater than 5% and only two patients had low haptoglobin concentrations.

It is concluded that while biochemical evidence of vitamin E abnormality is common in Crohns disease this does not appear to be of clinical significance.

**The effect of diet on bile acid and neutral steroid excretion in relation to the risk of colon cancer.** By M. S. SIAN and R. M. GREENHALGH, *Professorial Department of Surgery, Charing Cross Hospital Medical School, London W6 8RF*

There is increasing evidence to suggest that diet, bacteria and bile acids are important in the pathogenesis of colon cancer (Hill, 1977). Epidemiological studies show that the incidence of colon cancer is low in developing countries of Africa and Asia. It is suggested that the high proportion of dietary fibre consumed by these low incidence populations may be protective against this disease (Pomare & Heaton, 1973).

In studies of colon cancer, the analysis of bile acids and neutral steroids found in the faeces is essential but the methods available are rather complex and unsuitable for routine work. In the present study we have used a semi-automated technique for the extraction stage and have estimated the bile acid and neutral steroid excretion in subjects taking a normal, mixed diet and in those taking a non-meat, vegetarian diet.

Group A subjects ( $n$  16, age 30 (SD 7) years, body-weight 60 (SD 5) kg) ate a mixed diet. Group B subjects ( $n$  10, age 31 (SD 7) years, body-weight 57 (SD 5) kg) ate a vegetarian diet excluding meat and fish but not eggs. A 3-d stool sample was collected by all subjects and the faecal steroids analysed as described previously (Sian, 1982).

*Effect of diet on faecal bile acid and neutral steroid excretion*

Steroids (mg/g dry faeces)	Group A		Group B		Statistical significance $P \leq$
	Mean	SD	Mean	SD	
Neutral	17.5	4.9	12.2	2.2	0.01
Acidic	9.3	3.1	6.2	1.8	0.01
Total	26.8	6.7	18.4	2.6	0.001

Total bile acid and neutral steroid excretion was higher in subjects taking a mixed diet ( $P < 0.001$ ); both the acidic as well as the neutral steroid excretion in these subjects was significantly higher than that found in the vegetarians ( $P < 0.01$ ). Comparison of the faecal bile acid profiles showed that the bile acids were more extensively degraded in subjects taking a mixed diet than in those taking a vegetarian one. The higher excretion of the total faecal steroids in those taking a mixed diet may expose these subjects to a higher risk of colon cancer on the premise that certain steroids may act as promoters of cancer or carcinogens for the colon.

Hill, M. J. (1977). In *The Bile Acids*, vol. 3, pp. 190–192. New York: Plenum Press.

Pomare, E. W. & Heaton, K. W. (1973). *British Medical Journal* 4, 262–266.

Sian, M. S. (1982). The analysis of bile acids and neutral sterols in human faeces and the bile salts in human bile using some newly developed chromatographic techniques. M.Phil Thesis, University of London.

**Treatment of refractory obesity with a low-energy liquid diet—preliminary results.** By M. J. HALL<sup>1</sup>, D. GOODISON<sup>2</sup> and R. E. BARRY<sup>1</sup>,  
<sup>1</sup>University Department of Medicine and <sup>2</sup>Department of Dietetics, Bristol Royal Infirmary, Bristol BS2 8HW

Total starvation is an effective method of obtaining rapid weight loss but requires hospitalization and results in loss of lean body mass, ketosis and electrolyte upset which may be hazardous (Lawlor & Wells, 1969). Modified fasting seeks to overcome these disadvantages by providing a low-energy liquid diet of known composition as the only nutritional intake.

Eighteen women (mean age 37, range 16–74 years; mean weight 108.9, range 76.2–151.1 kg) took a liquid formula diet (Modifast, Wander) providing daily 1.74 MJ (410 kcal), 70 g protein, 30 g carbohydrate, vitamins and minerals for between 1 and 10 months. All women had previously failed to lose weight despite repeated supervised dietary restriction.

Follow-up was by fortnightly dietician's interview and monthly medical assessment where weight, biochemical and haematological indices were measured, and compliance determined. Maximum mean weight loss ( $n$  12) was 12.5 kg at 5 months and mean weight rose thereafter. Mean body mass index (weight/height<sup>2</sup>) fell by five during this period. There was no change in biochemical or haematological indices. Ten patients were withdrawn from the diet because of poor compliance (3), default (3), depression (2) and intercurrent illness (2). Comparison of treatment withdrawals with those continuing the diet are shown in the table.

Patients	$n$	Period of follow-up (months)		Total weight loss (kg)		Rate of weight loss (kg/month)
		Mean	Range	Mean	Range	Mean
Withdrawn	10	5.4	1–10	9.5	0.4–22.6	2.3
Continuing	8	5.2	1–9	10.5	2.2–25.3	3.6

None of the differences were statistically significant.

We conclude that low-energy liquid diet therapy has a high withdrawal rate, that there are no apparent differences between those withdrawn and those who persist and that maximum mean weight loss is achieved at 5 months.

Lawlor, T. & Wells, G. (1969). *American Journal of Clinical Nutrition* 22, 1142–1149.

**The folate status of pastoral Fulani communities in northern Nigeria.** By JOSEPH A. ABALAKA, RAZIA S. HUQ and WINIFRED L. STAFFORD\*,  
*Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria*

From analysis of the folate content of the foodstuffs of the Fulani people and assessment of their intake (Huq *et al.* 1983), it was considered that they may consume insufficient folate. This is substantiated by a report that megaloblastic anaemia is common in northern Nigeria (Oomen, 1975).

The folate content of the red blood cells (RCF) and serum (SFA) of pastoral Fulani villagers (twenty-eight men, fourteen women, thirty-two children) and fifty blood donors, townsmen who normally eat a more varied diet, was measured. The procedure of Fleming *et al.* (1971) using *Lactobacillus casei* was employed. Values for SFA below 2 ng/ml, generally taken as the lower limit of normal, were found in three blood donors and in two Fulani men, two women and eight children. Of these, two Fulani men and two children had values below the 95% confidence limit (1.48 ng/ml) derived for the blood donors.

A better assessment of folate status, over several months, can be made from the RCF values. The lower confidence limit for the donors was 173 ng/ml and twelve Fulani villagers, including eight (25%) of the children, had lower RCF values. We conclude that they form a group of inferior folate status, however, only three Fulani men and three children had RCF values below 160 ng/ml, the usual lower limit of normal. Statistical analysis of the RCF values for Fulani men and for the children showed lower confidence limits of less than 130 ng/ml; indeed two children had RCF values of less than 120 ng/ml.

We conclude that a proportion of the Fulani population have only marginally adequate folate status.

Fleming, A. F., Comley, L. & Stenhouse, N. S. (1971). *American Journal of Clinical Nutrition* **24**, 1257-1264.

Huq, R. S., Abalaka, J. A. & Stafford, W. L. (1983). *Journal of the Science of Food and Agriculture* **34**, 404-406.

Oomen, J. M. V. (1975). *East African Medical Journal* **52**, 208-218.

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**The influence of diet on intestinal mucin production.** By J. QUARTERMAN and LYNNE M. FEARNs, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The fasting of rats for 16–24 h, modification of the lipid content of their diet or the substitution of liquid milk for solid food markedly influence the absorption of transition elements and heavy metals (Quarterman & Morrison, 1980, 1981; Quarterman *et al.* 1977). As a prelude to investigating possible relationships between these phenomena, the effects upon mucin production of a variety of dietary treatments known to increase metal absorption have been investigated. Five groups of eight rats of mean weight *ca.* 100 g received the following dietary treatments: group C (control), a semi-purified diet containing 50 g arachis oil/kg; group HF, the basal diet with 200 g arachis oil/kg for 3 weeks; group L, the basal diet with 5 g lecithin/kg; group S, the basal diet but food withdrawn 16 h before killing; group M, the basal diet replaced by fresh bovine milk for 3 d. Each rat was given 750 kBq D-[1-<sup>3</sup>H]galactose intraperitoneally in 0.2 ml saline 90 min before killing under anaesthesia. The proximal 100 mm of the duodenum was removed from each rat and treated according to the method of Weiser (1973) to separate apical, middle and basal parts of the villi. Mucosa from the second 100 mm section of the duodenum was removed by scraping and fractionated into brush borders, a 40 000 g pellet and the remaining supernatant (cytosol). Each fraction was analysed for protein, hexosamine and radioactivity.

The total protein recovered in villus fractions from rats given treatments HF, L or M was reduced. Total hexosamine recovered was significantly increased in the middle parts of the villi by all treatments and in the 40 000 g pellet and mucosal cytosol by treatment M. The content of hexosamine ( $\mu\text{g}/\text{mg}$  protein) was increased by a factor of two or more in the villi of groups HF, L and M and in the cytosol by treatment M. Tissue activity expressed as counts/min per mg hexosamine was decreased in the apical and middle parts of the villi and in all cell fractions by all treatments.

Most of the hexosamine in intestinal mucosa is associated with mucous glycoproteins and in the first few hours after injection of a labelled hexosamine the label is found only in glycoproteins (Weiser, 1973). Our results therefore suggest that dietary manipulation can have a considerable effect on the small intestinal mucus. Assuming that treatments HF, L and S did not change the galactose pool, the results may indicate that the quantity of mucin was increased and its turnover decreased by these treatments.

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**The influence of diet on stool buffer capacity.** By S. J. ROSE, *Department of Child Health, University of Aberdeen, Medical School, Foresterhill, Aberdeen AB9 2ZD*

The advantages of breast feeding include the reduced incidence of sudden infant death syndrome, improved bonding with a reduction in the incidence of child abuse and improved immunological status with subsequent reduction in generalized infections and, specifically, gastroenteritis. Alteration in gut flora induced by different diets was postulated to be the reason for reduced gastroenteritis as lactobacilli predominate in breast-fed infants and coliforms in those artificially fed. Immune mechanisms are important in producing this difference, but the major biochemical factor influencing gut flora was postulated to be the buffering capacity ( $B = \frac{\Delta \text{base}}{\Delta \text{pH}}$ ) of the stool which was determined by the buffering capacity of the milk. The marked bacteriological effect of buffering capacity has subsequently not been confirmed, but scant attention has been paid to the possible importance of intestinal buffering capacity in man. In vitro work has demonstrated the potent effect of buffer systems on viral, cellular and drug metabolism. Viral adsorption on to cell membranes prior to invasion may be dependent on viral aggregation which is pH dependent. Platelet metabolism and adhesion is altered in different buffer systems and cellular handling of metal ions is similarly altered. Drug dissolution and intestinal transfer are both pH and buffer dependent, which has implications for bioavailability.

We decided to study the effect of diet on stool buffering capacity. Three groups of infants were established on different feeds: (1) breast ( $n$  9), (2) standard formula (SF; 18 g protein/l) ( $n$  6) and (3) low-protein formula (LPF; 15 g protein/l) ( $n$  7). One hundred and ten stool specimens were studied, portions of the stools were ultracentrifuged and biochemical assays performed on the resultant stool water. The buffering capacity curves of stool waters were determined and also different buffer systems were studied including protein, phosphate, lactate and bicarbonate. Buffering capacity of whole stools was determined.

Feed . . .	Buffering capacity (equivalents $\times 10^{-2}/l$ )					
	SF		LPF		Breast	
	Mean	SE	Mean	SE	Mean	SE
Milk	4.9	0.4	4.1	0.3	3.6	0.1
Stool water	13.5	0.9	12.3	0.1	14.4	0.6
Whole stool	83.6	5.1	42.3	3.8	37.8	4.6

The results were analysed by the Wilcoxon Rank Test. The only significant difference was in the buffering capacity of whole stools with the SF feed producing a significantly higher ( $P < 0.001$ ) buffering capacity than the other two feeds. It is suggested that much of the extra buffering capacity was due to the higher protein content of the SF feed compared with the LPF feed and breast milk. Thus, biochemical manipulation of the diet has a profound influence on gut biochemistry.

**Effect of soya-bean protein on chicken meat iron absorption.** By Y. LATUNDE-DADA and R. J. NEALE, *Department of Applied Biochemistry and Food Science, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough LE12 5RD*

Recently, there has been growing concern about the inhibitory effect of soya-bean protein on meat iron absorption. This is possibly due to the increasing use of soya-bean products as meat extenders and substitutes. Cook *et al.* (1981) reported a decrease in total meal Fe absorption in humans from 5.5 to 1.0, 1.9 and 0.4% when full fat soya-bean flour, textured soya-bean and soya-bean protein isolate respectively replaced egg albumin in a semi-synthetic meal. We have studied the effect of three soya-bean products of known protein and phytic acid contents on Fe absorption from intrinsically ( $^{59}\text{Fe}$ -labelled) chicken meat using the 2 h in vivo rat technique described by Bogunjoko *et al.* (1983). Soya-bean products replaced the chicken meat protein at two levels of protein substitution (30 and 50%) but all test meals contained the same level of total protein (500 mg). The results and analyses are shown in the table.

Test meal	% chicken protein replaced	Fe absorption (%)		Reduction in Fe absorption (%)
		Mean	SE	
Chicken meat	0	15.8	0.61	
Soya-bean product*:				
Defatted flour	50	7.60	0.35	51.8
	30	9.05	0.96	42.6
Concentrate flour	50	4.97	1.02	68.5
	30	6.15	0.48	61.0
Isolate	50	9.11	0.16	42.2
	30	11.4	0.92	27.9

\*Protein and phytic acid (g/kg) respectively in: defatted flour 493, 16.3; concentrate flour 674, 12.8; isolate 828, 13.0.

All soya-bean samples were inhibitory to chicken Fe absorption, the concentrate being most inhibitory while the isolate was least inhibitory. In general, higher levels of substitution gave larger reductions in chicken Fe absorption. Further work has shown that the inhibitory effect of the soya-bean concentrate is most pronounced on the haemosiderin, ferritin and low molecular weight fractions, but the haemoproteins are relatively unaffected.

We thank British Arkady, Manchester, for the soya-bean flour.

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**The complex origin of lymph phospholipids in the sheep.** By W. M. F. LEAT and F. A. HARRISON, *AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

The precise origin of lymph phospholipids, which account for 20–25% of total lymph lipids in the sheep, is still unclear. Leat & Harrison (1974) concluded that a large proportion of the lymph phospholipids in the sheep are of endogenous origin and could have been derived partly from bile. Further evidence has suggested that sources other than bile may contribute to the pool of lymph phospholipid (Leat & Harrison, 1977). In an attempt to extend these observations, experiments on sheep with biliary fistulation and re-entrant fistulation of the thoracic lymph duct have been performed.

Within 3 h of diversion of bile, lymph triglyceride (TG) and phospholipid (PL) concentrations decreased from 3680 to 696 mg/l and from 1095 to 400 mg/l respectively. The subsequent intraduodenal infusion of bile salt solution (50 g Oxoid bile salts/l isotonic sodium chloride–sodium bicarbonate buffer at 1 ml/min) increased the lymph concentrations of TG and PL to 1974 and 737 mg/l respectively after 2.5 h infusion. However, the response was not sustained and after 4 h infusion of bile salts the lymph TG and PL had returned to the pre-infusion bile-depleted levels.

When plasma lipids were radiolabelled by intravenous infusion of 50  $\mu$ Ci [ $1-^{14}$ C]linoleic acid (see Lindsay & Leat, 1977), the distribution of radioactivity in plasma after 24 h was 36.6 nCi/l in 101.7 mg TG/l, and 372 nCi/l in 875 mg PL/l. The concomitant values in thoracic duct lymph were much greater at 503.3 nCi/l in 6821 mg TG/l and 1308 nCi/l in 2320 mg PL/l. These observations suggest that radiolabelled bile phospholipids synthesized in the liver were secreted into the gut lumen and incorporated into lymph PL and TG after intraluminal hydrolysis and absorption. Diversion of bile, which was radioactive, followed immediately with fluid replacement by intraduodenal infusion of isotonic NaCl–NaHCO<sub>3</sub> buffer caused decreases in the radioactivity and concentrations of lymph PL and TG to less than 10% of the pre-diversion levels. Subsequent intraduodenal infusion of the bile salt solution resulted in an increased secretion into the lymph of  $^{14}$ C-labelled TG and PL. The most probable source of this radiolabelled lipid seems to be the pool of PL within the intestinal mucosa which, under normal circumstances, is replenished by PL derived from sources such as bile and plasma.

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**Lipid absorption in sheep with two types of biliary fistulation.** F. A. HARRISON and W. M. F. LEAT, *AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

There is strong evidence to indicate that bile is essential for the absorption of lipid into the lymphatics of the sheep, but the evidence of a role for pancreatic juice is less convincing (Heath & Morris, 1964; Harrison & Leat, 1972). The separate secretions of bile and pancreatic juice can be obtained in the sheep by surgically diverting bile to the upper jejunum which allows the collection of pancreatic juice by transduodenal catheterization of the common bile and pancreatic duct (see Harrison, 1979). After cannulation of the thoracic duct in three sheep with this type of biliary fistulation, it was noted that the lymph was only intermittently milky whereas it is usually permanently milky because of the continuous nature of the digestive process in ruminant animals. After this type of surgery, pancreatic juice is mixed with acidic digesta from the abomasum before the addition of bile; in unoperated animals of any species bile enters the duodenum cranial to or with pancreatic juice.

The surgical procedure was therefore modified so that bile was diverted into the duodenum cranial to the pancreatic duct (see Harrison, 1979) and after this type of biliary fistulation thoracic lymph was permanently milky. Since one of the lipolytic enzymes in sheep pancreatic juice is sensitive to acidity in the pH range 2.5–3.5 (Arienti *et al.* 1974) and the pH of abomasal digesta ranges from 2.1–3.3 (Harrison & Hill, 1962), inactivation of the enzyme to varying degrees could account for the intermittent absorption of lipid with the first type of biliary fistulation, and could provide indirect evidence of a role for pancreatic juice in lipid absorption in sheep.

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**The effect of re-entrant fistulation of the ileum on the heat loss of adult sheep.** By W. H. CLOSE,\* F. A. HARRISON and R. P. HEAVENS, *AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

Re-entrant fistulation of the intestine requires the diversion of the digesta through inert tubing to bring the flow out of and back into the intestine and a small requirement of energy for this process is to be expected. However, MacRae *et al.* (1982) found significant increases in heat production by sheep after double re-entrant fistulation of the duodenum and ileum. Close *et al.* (1984) reported that the metabolism of sheep was not significantly altered after rumen and single duodenal re-entrant fistulation and we now report studies on the effect of single ileal re-entrant fistulation with rumen fistulation.

Four 2 year-old Clun Forest ewes were fed and maintained under similar experimental conditions to those described by Close *et al.* (1984) and observations of 24 h energy expenditure made for 7-d periods in a direct heat-sink calorimeter (Close & Mount, 1975) at an environmental temperature of  $20 \pm 0.1^\circ$ . After pre-operation measurements of heat loss, each animal was surgically prepared with a rumen fistula and, 9 weeks later, a single re-entrant fistula of the ileum approximately 150–200 mm from the ileo-caecal junction, except in one animal where the fistula was from the ileum to the caecum. All surgery was performed under general anaesthesia and T-shaped Perspex ileal cannulas with an internal diameter of 16 mm were used. The results are summarized in the table.

*Heat loss of adult sheep (MJ/d) (n 4)*

Weeks following:						
Rumen fistulation	–0.05	2.5	7.5	11.5	15.5	28.5
Ileal fistulation	—	—	—	2.5	6.5	19.5
Heat loss:						
Mean	7.85	7.92	7.61	7.89	7.63	7.56
SEM	0.3	0.4	0.2	0.2	0.2	0.3

There was no significant change in the absolute rates of heat loss ( $P > 0.05$ ) for up to 20 weeks after ileal surgery. Unlike the situation in duodenal preparations (Close *et al.* 1984), there were occasional blockages of digesta which caused reductions in appetite and body-weight. Autopsies on three sheep 8 months after ileal surgery revealed no gross pathology, although there was some thickening of the ileal wall cranial to the fistulation. The fourth sheep was then given a daily supplement of 100 g concentrate nuts (140 g protein/kg) until its body-weight returned to the pre-surgery level. Subsequently, body-weight has been maintained and not any blockage of digesta flow has occurred for over 4 months.

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**Dietary intakes of three ethnic groups of Singapore.** By PUI YONG TAN, T. B. NG and N. SAHA, *Department of Physiology, Faculty of Medicine, National University of Singapore, Singapore 0511*

The mortality rate in Singapore due to coronary heart disease in Indians is about four times that of Chinese or Malays (Singapore Registrar-General, 1980). This has prompted this study for the identification of possible differences in the dietary intake of these groups.

528 Male employees of the Port of Singapore Authority, from three ethnic groups of comparable age and socio-economic status, were investigated for their dietary habits and nutritional status by a 24-h dietary recall method. Daily intakes of nutrients including vitamins and minerals were computed from food composition tables (Platt, 1962; Leung, 1972).

*Nutrient intakes of Chinese, Malays and Indians*

	Chinese (n 205)		Indians (n 167)		Malays (n 156)		Total (n 528)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-weight (kg)	60.7	0.67	64.1	0.80	61.5	0.90	62.0	0.45
Ponderal index*	42.2	0.14	41.2	0.15	41.5	0.18	41.7	0.09
Energy (MJ)	9.16	0.07	9.31	0.08	9.26	0.10	9.24	0.05
Carbohydrate (g)	337.5	3.12	362.4	4.07	374.0	4.59	356.1	2.32
Protein (animal) (g)	29.2	0.70	24.1	0.69	22.8	0.74	25.7	0.43
Fats (g)	63.2	0.9	58.2	1.1	53.3	1.1	58.7	0.60
Polyunsaturated fats (g)	13.5	0.29	12.1	0.30	12.4	0.39	12.7	0.19
Cholesterol (mg)	448.4	13.6	376.0	16.1	356.4	17.5	398.3	9.10
Calcium (mg)	671.6	19.6	843.0	26.1	726.7	25.1	742.1	13.80
Thiamin (mg)	1.7	0.03	1.0	0.02	0.8	0.02	1.2	0.02
Nicotinic acid (mg)	19.0	0.31	11.9	0.21	11.5	0.21	14.5	0.22

\*Height<sup>3</sup>/weight.

The Indian subjects had a significantly higher ( $P < 0.05$ ) mean body-weight (64.1 kg) and lower ( $P < 0.001$ ) ponderal index (41.2) than Chinese (60.7 kg; 42.2) and Malays (61.5 kg; 41.5).

The results in the Table show a significantly lower intake of calcium in Chinese as compared with Indians ( $P < 0.001$ ). The Chinese, however, had higher ( $P < 0.001$ ) intakes of animal protein, nicotinic acid and thiamin. Their intakes of both polyunsaturated fats and cholesterol were also significantly higher ( $P < 0.05$ ). The intake of carbohydrates was significantly lower ( $P < 0.001$ ) in Chinese than in Indians and Malays. No significant difference in other intakes in these groups was noted. The intakes of pork, pig's liver, duck and prawn were higher in Chinese compared with those of Indians who consumed mainly mutton. There are, however, striking differences in their cooking methods and in the use of cooking media. Indians use coconut oil and ghee while the Chinese favour corn oil and lard. The Malays prefer coconut oil.

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