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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Thirty-second Meeting of the Nutrition Society (One Hundred and Seventy-first of the Scottish Group) was held in the Pollock Halls, University of Edinburgh on Wednesday and Thursday, 24/25 September 1986, *when the following papers were read:*

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The effects of fasting and fibre supplements on some metal-binding properties of rat intestinal mucus. By J. QUARTERMAN, J. H. ANDERSON and ALISON MCDONALD, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The fractional absorption of a number of metals given orally is increased if the animal or human subject is fasted for 16–24 h before the dose of metal (Quarterman & Morrison, 1981). After fasting, the amount of metal associated with intestinal mucus increased (Quarterman, 1985) and the present report describes increased ^{65}Zn binding to one fraction of this mucus.

Three groups of twelve rats, weighing 125–150 g, were given a semi-purified diet for 7 d or this diet supplemented with 100 g pectin or 100 g cellulose/kg. Half of each group were starved for 16 h, then each rat was given 3 μCi ^{65}Zn, 5 μg total in 0·2 ml saline (9 g sodium chloride/l) by stomach tube. The rats were anaesthetized 2 h later and a 200 mm length of duodenum–jejunum washed out and filled with agarose solution (30 g/l). The agarose occludes mucus without damaging the mucosa. The mucus was eluted from the agarose and fractionated on Sepharose 6B into two major fractions. Each fraction was analysed for its absorption at 280 nm, its glycoprotein content and its ^{65}Zn activity. One fraction eluting at the void volume probably contained high-molecular weight mucus glycoprotein and had very little ^{65}Zn. Most of the activity appeared in the broad fraction of about 80000 daltons molecular weight (MW).

The concentration of ^{65}Zn (counts/min in the mucus fraction per unit absorbance of glycoprotein) in the lower MW fraction was increased by fasting by a factor of 1·5–2·0, and the amount of this fraction present (unit absorbance of glycoprotein/mm gut length) was also increased by a similar factor. Fibre supplementation did not affect either index. The amount of the higher-MW fraction and its ^{65}Zn content were not affected by fasting, but fibre supplementation decreased the amount of this fraction/mm gut length in fed but not fasted rats.

Fasting thus changed the composition of intestinal mucus in such a way as to increase its metal-binding properties. This may be related to the increased sialic acid content observed after fasting and may be a factor in the increased absorption of metals after fasting. The mucus layer is the first part of the gut to come into contact with digesta and an increased capacity of this layer to take up metals can render more of the metal available to the absorbing surface of the mucosa.

Quarterman, J. (1985). In *Trace Elements in Man and Animals*—5 pp. 400–401 [C. F. Mills, I. Bremner and J. K. Chesters, editors]. Slough: Commonwealth Agricultural Bureaux.

Quarterman, J. & Morrison, E. (1981). *British Journal of Nutrition* **44**, 277–287.

The effect of dietary fibre on apparent absorption of zinc, copper, iron and manganese in the elderly. By MARGARET S. LAWSON, *Department of Food and Biological Sciences, Polytechnic of North London, Holloway Road, London N7*, and VALDA W. BUNKER and BARBARA E. CLAYTON, *Chemical Pathology and Human Metabolism, Southampton General Hospital, Southampton*

Metabolic balance studies were carried out for 5 d on twelve healthy elderly men and women living in their own homes and eating their normal food. The zinc, copper, iron and manganese contents of the diet and faeces were analysed and the dietary fibre content calculated. The mean fibre intake was 18·9 g/d (range 6·5–30·2 g/d); mean intakes of Zn, Fe, Cu and Mn were 144, 202, 24 and 96 μmol/d respectively. Correlation coefficients were calculated for each of the variables.

Correlations between fibre intakes and trace element intakes and absorption

(Values in parentheses are correlation coefficients for cereal fibre and percentage cereal fibre intakes respectively)

	Intake	Quantity absorbed	Percentage apparent absorption
Zn	+0·69***	+0·48	+0·42 (−0·02, −0·41)
Cu	+0·51*	+0·18	+0·24 (−0·03, −0·27)
Fe	+0·84***	+0·52*	+0·51* (−0·03, −0·62*)
Mn	+0·86***	+0·40	+0·56* (+0·63*, −0·31)

*$P \leqslant 0{\cdot}05$, ***$P = 0{\cdot}001$.

Despite the positive correlation between the fibre and trace metal contents of the diet, there was no significant relation between total fibre intake and either the total quantity or percentage of dietary Zn and Cu apparently absorbed; higher intakes of fibre appeared to be associated with higher total Fe absorption and an increase in percentage of dietary Fe and Mn apparently absorbed. The type of fibre, however, did appear to exert an effect: there was a trend towards a decreased apparent absorption of Zn, Cu and Fe with higher intakes of cereal fibre, though this did not hold true for Mn. Diets which contained a higher proportion of cereal fibre seemed to result in lower percentage apparent absorption of metals, though this was only significant for Fe.

Several factors may account for the apparent lack of significant effect of dietary fibre on apparent trace metal absorption: the levels of fibre consumed may not have been sufficiently high; in addition, some adaptation may have occurred in the subjects consuming large quantities of fibre, since they had been eating this type of diet for several years. A high percentage of the total fibre intake (mean 53%) came from vegetable fibre and our results suggest that cereal fibre may exert a greater inhibitory effect on apparent absorption than non-cereal fibre.

Na^+,K^+-ATPase-dependent zinc transfer by everted gut sacs from rats given different amounts and types of dietary fibre. By C. J. SEAL and J. C. MATHERS, *Department of Agricultural Biochemistry and Nutrition, The University, Newcastle upon Tyne NE1 7RU*

Impaired mineral nutrition is a possible adverse effect of increased consumption of foods rich in dietary fibre (DF) (Royal College of Physicians, 1980) but several recent studies have found no effects on mineral balance in man of substantial increases in DF intake. Whilst some types of DF may bind minerals and reduce their rates of absorption from the small intestine, it is possible that these minerals are released when the DF is fermented in the large intestine (Cummings *et al.* 1979) but little is known of the capacity of the distal intestine to absorb minerals other than the common electrolytes. We have measured rates of zinc transport into everted gut sacs from the duodenum (D), ileum (I) and colon (C) of rats given different amounts and types of DF.

Rats (initial weight 220 g) were offered one of four diets: (1) stock or semi-purified diets based on that of Seal & Heaton (1983) containing (2) no fibre, (3) 200 g wheat bran/kg or (4) 200 g citrus pectin/kg. After 25 d, the animals were killed for in vitro measurements (Seal & Heaton, 1983) of Zn transfer (μg Zn/g tissue dry weight per 30 min) by D, I and C sacs in the presence and absence of 0·25 mM-ouabain to estimate Na^+,K^+-ATPase-dependent Zn transfer.

		D			I			C		
Diet	No. of animals	−o	+o	o dep	−o	+o	o dep	−o	+o	o dep
(1) Stock	6	6·6	5·2	1·4	9·0	8·2	0·8	12·4	9·2	3·2
(2) No fibre	6	7·4	4·8	2·6	8·3	7·8	0·5	7·6	3·1	4·5
(3) Bran	4	9·1	5·8	3·5	11·6	10·4	1·3	12·6	11·1	1·6
(4) Pectin	6	14·6	9·5	5·1	15·3	12·9	2·4	16·1	6·6	9·5

−o, no ouabain; +o, ouabain present; o dep, Na^+,K^+-ATPase-dependent; SE of mean 1·5 (n 6).

Rates of Zn transfer into sacs from bran- or pectin-fed rats were higher than those from no fibre animals. There were no significant ($P>0·05$) differences between intestinal sites in rates of Zn transfer but sites were ranked in the order C>I>D. Inclusion of ouabain reduced significantly ($P<0·001$) rates of Zn transfer at all sites regardless of diet. On average, 0·29 of Zn transfer was Na^+,K^+-ATPase-dependent but there were considerable between-site differences. Ouabain inhibited 0·38 and 0·33 of Zn transfer with C and D sacs respectively but only 0·11 with I sacs. The colon appears to have considerable capacity to absorb Zn by Na^+,K^+-ATPase-dependent mechanisms especially in animals given fermentable DF such as pectin.

Cummings, J. H., Southgate, D. A. T., Branch, W. J., Wiggins, H. S., Houston, H., Jenkins, D. J. A., Jivraj, T. & Hill, M. J. (1979). *British Journal of Nutrition* **44**, 477–485.

Royal College of Physicians (1980). *Medical Aspects of Dietary Fibre.* Tunbridge Wells: Pitman Medical.

Seal, C. J. & Heaton, F. W. (1983). *British Journal of Nutrition* **50**, 317–324.

Selenium concentration and glutathione peroxidase activity in human blood from patients with coronary heart disease. By M. N. I. BARCLAY, A. MACPHERSON, C. TAYLOR and W. H. R. AULD, *West of Scotland College of Agriculture, Ayr KA6 5HW*

Symptoms of selenium deficiency and their reversal with Se supplementation in domestic livestock are well documented (Underwood, 1977) and a metabolic role for Se as a constituent of glutathione peroxidase (*EC* 1.11.1.9; GSHPx) has been elucidated (Awasthi *et al.* 1975). The level of Se in human blood has been shown to vary greatly depending on country and diet (Schrauzer & White, 1978). However, within the same area, association has been shown between 'cardiovascular death, myocardial infarction and blood selenium levels' (Solonen, 1982). The present study was designed to measure blood Se levels and GSHPx activity in coronary heart disease (CHD) patients and a similar number of control surgical patients.

Blood Se, measured by a modification of the fluorimetric procedure of Olson (1969), showed a range of 12–186 μg/l with a mean of 119 μg/l in samples taken from 354 patients from North Ayrshire. When divided into CHD samples and controls there was no significant relation between CHD and Se concentration in the whole blood.

A positive correlation has been shown between GSHPx activity in the whole blood of cattle and Se status (Anderson *et al.* 1978). No such correlation was shown in the present study on human whole blood where the GSHPx activity ranged from 1 to 29·8 with a mean of 8·16 u/ml. However, blood samples from CHD patients showed significantly ($P<0.001$) lower GSHPx activity than those from controls. No significant correlation was found between age of the patients and their blood Se level or GSHPx activity but there was a higher GSHPx activity in samples from females with CHD (see Table).

Blood GSHPx activity (u/ml)

	CHD		Controls	
	Mean	SE	Mean	SE
♂	5·768	0·536	10·069	0·666
♀	7·288	0·609	9·045	0·547
Total	6·544	0·410	9·569	0·433

Anderson, P. H., Berrett, S. & Patterson, D. S. P. (1978). *Journal of Comparative Pathology* **88**, 181.

Awasthi, V. C., Beutler, E. L. & Scrivastava, S. K. (1975). *Journal of Biological Chemistry* **240**, 144–149.

Olson, O. E. (1969). *Journal of the Association of Official Agricultural Chemists* **52**, 627–630.

Schrauzer, G. N. & White, D. A. (1978). *Bio-inorganic Chemistry* **8**, 303–318.

Solonen, J. T. (1982). *Lancet* **ii**, 175.

Underwood, E. J. (1977). *Trace Elements in Human and Animal Nutrition*, 4th ed, pp. 302–340. London: Academic Press.

Effect of maternal zinc supply on metallothionein levels in blood and liver of rat pups. By J. N. MORRISON and I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It has been suggested that measurement of plasma metallothionein (MT) concentrations can be of value in the diagnosis of zinc deficiency (Sato *et al.* 1984). The likelihood of Zn deficiency problems arising in humans is particularly great during the perinatal period, when demand for Zn is high and maternal Zn supplies may be limited. Since liver and plasma MT concentrations are greatly increased in neonatal rats (Mehra & Bremner, 1984), a study has been made of the changes in MT levels in blood and liver of rat pups from dams given diets of varying Zn content.

Three groups of twelve nulliparous female Hooded Lister rats were mated, and after 1 week were given a semi-synthetic diet containing 6, 9 or 40 mg Zn/kg. These diets were given for the remainder of the gestation period and during lactation. Litters were culled to eight pups/litter at 1 d post-partum. At 7, 14 and 21 d of lactation, four litters from each group were killed and liver and heparinized blood were collected. Concentrations of MT-I in plasma, lysed blood cells and in the liver were measured by radioimmunoassay (Mehra & Bremner, 1983).

		Dietary Zn content (mg/kg)					
	Period of experiment	6		9		40	
MT-I in:	(weeks)	Mean	SEM	Mean	SEM	Mean	SEM
Liver (μg/g)	1	126	9	231	14	302	25
	2	44	7	57	4	98	10
	3	5	1	12	1	13	2
Plasma (ng/ml)	1	69	11	178	24	472	99
	2	22	3	18	2	32	5
	3	4	1	12	2	12	1
Blood cells (μg/g haemoglobin)	1	3·8	0·3	5·9	0·7	5·7	0·6
	2	2·2	0·6	1·5	0·2	3·4	0·5
	3	0·4	0·1	1·9	0·2	2·8	0·4

Although there was no effect of maternal Zn supply on the weight of the pups at 1 week, at 2 and 3 weeks the pups from dams given the diet containing 6 mg Zn/kg weighed about 15% less than the others. At 3 weeks the weights (mean and SEM) were 46·1 (0·1), 52·7 (0·9) and 52·6 (0·9) g for diets containing 6, 9 and 40 mg Zn/kg respectively.

In the Zn-adequate pups from dams given 40 mg Zn/kg there was a rapid decrease with age in MT-I concentrations in the liver and plasma and, to a lesser extent, in the blood cells. Decreasing the maternal Zn supply caused a further and significant reduction in these concentrations, especially in the litters from dams given 6 mg Zn/kg. Maternal Zn supply is therefore a major determinant of MT-I concentrations in neonatal tissues and assay of the levels of this protein in blood plasma or cells should give a useful indication of Zn status.

Mehra, R. K. & Bremner, I. (1983). *Biochemical Journal* **213**, 459–465.
Mehra, R. K. & Bremner, I. (1984). *Biochemical Journal* **217**, 859–862.
Sato, M., Mehra, R. K. & Bremner, I. (1984). *Journal of Nutrition* **114**, 1683–1689.

Metallothionein levels in blood cells and urine of zinc-deficient and endotoxin-treated rats. By I. BREMNER, J. N. MORRISON and ANNE M. WOOD, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Concentrations of metallothionein (MT) in plasma are reduced to non-detectable levels in zinc-deficient rats whereas they are increased in animals subjected to stress (Sato *et al.* 1984). Assay of plasma MT can therefore be used to distinguish between these causes of hypozincaemia and so aid the diagnosis of Zn deficiency. MT also occurs in the urine and blood cells of normal rats. A study has therefore been made of the effects of Zn deprivation and of endotoxinaemia on MT levels in urine and blood cell lysates.

Three groups of sixteen male Hooded Lister rats, aged 5 weeks, were given a semi-synthetic diet containing 3, 6 or 12 mg Zn/kg. At intervals of 3–4 d, four rats from each group were transferred to metabolism cages for 2 d for collection of urine. The rats were then killed and heparinized blood was collected. Concentrations of MT-I in the urine and in lysates of the blood cells were measured by radioimmunoassay (Mehra & Bremner, 1983). Groups of five rats maintained on stock diet were also injected intraperitoneally with endotoxin (1 mg/kg body-weight) and urine and blood cells collected at intervals over the following 48 h.

Concentrations of MT-I in both the urine and blood cells of the rats given the low-Zn diets were significantly reduced within 3–7 d; with 3 mg Zn/kg diet, blood cell MT-I levels were only 5–10% of those in rats given 12 mg Zn/kg; with 6 mg Zn/kg, they were 20–50% of control values. Urinary MT-I concentrations in both groups of Zn-deficient rats were only about 30% of those in control animals.

		Dietary Zn content (mg/kg)					
		3		6		12	
MT-I in:	Period of experiment (d)	Mean	SEM	Mean	SEM	Mean	SEM
Blood cells (ng/mg haemoglobin)	3	192	25	436	37	659	30
	7	32	16	137	20	614	53
	14	31	16	123	11	265	23
Urine (ng/mg creatinine)	3	170	50	100	8	161	10
	7	44	5	59	5	177	20
	14	45	7	46	2	126	21

Injection of endotoxin increased urinary MT-I concentrations (mean and SEM) from 73 (3) ng/mg creatinine to 661 (103) and 476 (49) ng/mg creatinine after 24 and 48 h respectively. Concentrations were restored to control levels after 96 h. However, injection of endotoxin had no significant effect on blood cell MT-I concentrations. These were 385 (32) ng/g haemoglobin before injection and 482 (59) and 468 (82) ng/g haemoglobin at 24 and 48 h post-injection. Assay of MT concentrations in urine and in blood cells can therefore also provide useful information on Zn status and, since basal levels are much greater than those in plasma, may offer certain advantages over analysis of plasma.

Mehra, R. K. & Bremner, I. (1983). *Biochemical Journal* **213**, 459–465.
Sato, M., Mehra, R. K. & Bremner, I. (1984). *Journal of Nutrition* **114**, 1683–1689.

Zinc deficiency, amino acid imbalance and brain catecholamine concentrations in the chick. By LYDIA HARRISON and J. P. F. D'MELLO, *Department of Agricultural Biochemistry, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG*

The feeding of zinc-deficient diets may increase brain concentrations of norepinephrine (NE) in the rat (Wallwork *et al.* 1982). Reeves & O'Dell (1984) found no such effect, but reported that reduction of the tyrosine and phenylalanine contents of a Zn-deficient diet increased food intake, this increase coinciding with a fall in the concentrations of NE and dopamine (DA) in the anterior hypothalamus. The current study was designed to investigate the effect of Zn deficiency on growth, food intake and brain concentrations of NE and DA in chicks fed on diets which were deficient in tyrosine and phenylalanine. A dietary amino acid (AA) imbalance was imposed by the addition of an AA mixture devoid of tyrosine and phenylalanine. The results are presented in the Table.

Supplement†	Catecholamine (ng/g fresh wt brain tissue) NE	DA	Mean wt gain (g/d per chick)	Dry matter intake (g/d per chick)
None	181	189	5	16
Imbalancing AA mixture‡	109	158	2*	11***
Imbalancing AA mixture + phenylalanine (5 g/kg)	166	180	14***	23***
Imbalancing AA mixture + phenylalanine (10 g/kg)	174	198	15***	25***
Zn acetate (0·17 g/kg)	200	290	6	19*
Zn acetate + imbalancing AA mixture	219	286	−0·5***	8***
Zn acetate + imbalancing AA mixture + phenylalanine (5 g/kg)	218	293	15***	25***
Zn acetate + imbalancing AA mixture + phenylalanine (10 g/kg)	290*	365***	20***	30***
SEM	29*	38	0·8	0·7
df	18	18	21	21

Mean values were significantly different from those for the unsupplemented diet: *$P<0·05$, ***$P<0·001$.

†Feeding period 7–21 d of age, chicks killed at 21 d of age.

‡Contains (g/kg diet): threonine 7·4, glycine 14, valine 9·8, methionine 9·2, isoleucine 8·5, leucine 14·7, lysine hydrochloride 13·8, histidine hydrochloride 6·0, arginine hydrochloride 12·4, tryptophan 2·1.

Zn supplementation tended to raise brain catecholamine concentrations. Phenylalanine supplementation only increased brain catecholamines in the presence of Zn, the effect being significant at the higher level of phenylalanine supplementation ($P<0·05$). Chicks fed on the imbalanced diet showed significantly greater growth ($P<0·05$) and intake ($P<0·01$) when that diet was also deficient in Zn.

L. H. acknowledges receipt of an AFRC research assistantship.

Reeves, P. G. & O'Dell, B. L. (1984). *Journal of Nutrition* 114, 761–767.
Wallwork, J. C., Botnen, J. H. & Sandstead, H. H. (1982). *Journal of Nutrition* 112, 514–519.

Differences in the zinc content of different regions of the toe-nail. By GRAHAME J. LAVIS, *Chelsea School of Chiropody, 18 Samford Street, London NW8 8EN* and VIDA OFEI and DAVID A. BENDER, *Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 7PN*

As part of a study of the relation between zinc nutritional status and wound healing, we have investigated the distribution of Zn in different regions of the toe-nail. Samples of nail from the great toe were obtained from twenty patients undergoing partial nail avulsion as treatment for ingrown or ingrowing toe-nail. Both sides of the nail, from the germinative matrix (nail-bed) to the distal end, were removed, resulting in a medial sample (from the side of the toe nearest the mid-line of the body) and a lateral sample (from the side adjacent to the second toe). The samples were cut into sequential strips from the distal to the nail-bed end, each weighing between 10 and 20 mg. Zn was determined by atomic absorption spectrometry after wet ashing in concentrated nitric acid.

Zn content of toe-nails (μ*mol/mg tissue, n 20*)

	Lateral aspect			Medial aspect		
	Mean	SD	Range	Mean	SD	Range
Nail-bed	4·1	1·78	2·5– 8·5	4·7	1·72	2·7– 8·0
Distal segment	5·1	2·45	3·3–12·0	3·9	1·32	2·3–11·1

The distal lateral portion of the nail contained significantly more Zn than other regions. The ratio of Zn in the distal:nail-bed sample from the lateral aspect was >1 in sixteen patients and <1 in only two. The ratio of Zn in the lateral:medial sample from the distal nail was >1 in the fifteen patients and <1 in only one. There were highly significant differences ($P<0.005$ by the Wilcoxon matched-pairs test) between the Zn content of lateral and medial distal samples of the same nail (but not for nail-bed samples) and between distal and nail-bed samples taken from the lateral, but not medial, aspect of the nail.

It thus appears that the toe-nail gains Zn during its growth from the germinative matrix to the distal end. Since the difference was more marked on the side adjacent to the second toe than on the 'open' side of the great toe, it is likely that this Zn is absorbed from sweat. In view of these results it is suggested that measurement of the Zn content of (distal) toe-nail clippings may not provide a useful indication of body Zn content and hence Zn nutritional status.

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Copper deficiency and antioxidant status in male and female rats. By S. M. LYNCH, D. G. M. CARVILLE and J. J. STRAIN, *Biomedical Sciences Research Centre, University of Ulster at Jordanstown, Newtownabbey, Co. Antrim BT37 0QB*

Free-radical-mediated peroxidation of membrane lipids may be involved in atherosclerosis and other diseases of affluence. The present study investigated the activities of antioxidant enzymes in tissues of copper-deficient male and female rats.

Weanling, Wistar rats of both sexes were housed individually and provided *ad lib.* with either (D) Cu-deficient (<0·4 mg Cu/kg) or (C) control diets for 10 weeks. The diets were based on those of Klevay (1973) but with 200 g maize oil/kg.

	Males				Females			
	C (n 5)		D (n 5)		C (n 5)		D (n 5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Leucocyte count (10^9)	4·04	1·12	7·88*	2·94	5·36	2·51	5·42	1·46
Erythrocyte count (10^{12})	3·82	1·48	2·70	0·75	5·20	1·34	5·1	0·41
Haemoglobin (Hb, g/l)	222	30	193	37	154	21	139	40·9
Erythrocyte: SOD (U/mg Hb)	1·83	0·48	1·52	0·86	0·49	0·48	0·25	0·2
Cat (U/mg Hb)	0·082	0·06	0·077	0·05	0·015	0·006	0·018	0·009
Macrophage SOD ($U/10^7$ cells)	1·20	1·36	2·60	2·04	4·80	1·84	1·62*	1·21
Liver: SOD (U/mg protein)	12·0	3·0	5·0*	2·0	3·0	2·0	1·0*	0·6
Cat (U/mg protein)	1·66	0·37	1·72	0·23	0·72	0·22	0·74	0·24
Glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) (U/mg protein)	0·023	0·007	0·05*	0·009	0·018	0·006	0·057*	0·018
Total cholesterol (mM)	2·10	0·15	3·90*	1·83	1·8	0·36	1·65	0·41
Low-density-lipoprotein cholesterol (mM)	1·60	0·10	3·10*	1·51	1·65	0·29	1·61	0·30
Triglyceride (mM)	1·78	1·10	2·80	1·82	0·726	0·28	0·95	0·59

Significantly different from controls: *P<0·05.

Granulocyte Cu/Zn- superoxide dismutase (*EC* 1.15.1.1; SOD) was significantly lower (P<0·01) in Cu-deficient male rats (C, 19·7 units/10^7 cells; D, 7·3 units/10^7 cells). Catalase (*EC* 1.11.1.6; Cat) and glutathione peroxidase (*EC* 1.11.1.9) activities were not significantly different in Cu deficiency although both C and D male rats had significantly higher (P<0·05) liver and erythrocyte Cat than females. Other sex-dependent influences on enzyme activities included significantly higher (P<0·05) liver and erythrocyte SOD in male rats and significantly higher (P<0·01) macrophage SOD in females. Liver total β-oxidation, peroxisomal β-oxidation and lactate dehydrogenase (*EC* 1.1.1.27/28) activity were not significantly different in Cu-deficiency. Under the conditions of this study, only male rats exhibited elevated serum lipid levels with Cu-deficiency.

S. M. L. acknowledges receipt of a Northern Ireland Chest, Heart and Stroke Association studentship.

Klevay, L. M. (1973). *American Journal of Clinical Nutrition* **26**, 1060–1068.

Belfast MONICA project first population survey: cholesterol, zinc, copper and selenium. By DOROTHY MCMASTER[1], A. E. EVANS[1], EVELYN E. MCCRUM[2], M. MACF. KERR[2] and C. C. PATTERSON[2], [1]*Department of Medicine and* [2]*Department of Community Medicine, The Queen's University of Belfast, Belfast BT12 6BJ*

The World Health Organization Coordinated MONICA project is the largest health study ever undertaken and involves forty centres throughout the world. The project aims to study incidence trends for fatal and non-fatal coronary heart disease over a 10 year period and to screen large, random, cross-sections of the population to establish risk-factor levels. This will take place on three occasions. The key factors to be assessed are serum cholesterol, high-density-lipoprotein cholesterol, blood pressure and smoking habits.

Age standardized mortality rates for 1984 reveal that Northern Ireland remains at the top of the world mortality league for coronary heart disease. The Belfast MONICA project includes several optional studies related to diet, health knowledge, various biochemical, electrocardiographical and psychosocial factors, and some measurements of trace elements. Any possible changes in the levels of these factors will be used to test the hypothesis that changes across the three surveys will be reflected in changes in the incidence of coronary heart disease.

The first population survey has been completed and the data are being analysed. The screening took place between October 1983 and September 1984 and a total of 2361 subjects were interviewed. The overall response rate was 64% and the age range 25–64 years. In addition to the obligatory measurements of cholesterol we have measured serum zinc and copper, whole blood glutathione peroxidase (*EC* 1.11.1.9) as an estimate of selenium status, serum thiocyanate as an indicator of smoking habit and γ-glutamyl transferase (*EC* 2.3.2.2) as an indicator of alcohol consumption.

The mean cholesterol level in males was 5·87 (SE 0·033) mmol/l and in females 5·90 (SE 0·036) mmol/l. Taking 6·70 mmol/l as the upper limit of normal, 21·5% males and 23·6% females were defined as having abnormal levels. To date we have noted that there is an increase in the ratio of copper:zinc with age in males but not in females and that the level of whole blood glutathione peroxidase is higher in females than males. The data are being analysed further. The interrelations between the results of our trace-element studies and some of the estimates of recognized risk factors for cardiovascular disease will be presented and discussed.

Estimation of leucocyte zinc and serum Zn and copper in geriatric long-stay patients. By WINIFRED STAFFORD, *Department of Biochemistry, University of Edinburgh, Edinburgh EH8 9YL*, and R. G. SMITH[1], ENID C. HENERY[2] and SUSAN LEWIS[2], [1]*Department of Geriatric Medicine and* [2]*Department of Nutrition and Dietetics, City Hospital, Edinburgh EH10 5SB*, and K. O'RORKE, *Department of Clinical Chemistry, Royal Infirmary, Edinburgh EH3 9YW*

Zinc in leucocytes, isolated using Ficoll in an adaptation of the method of Hinks *et al.* (1982), serum zinc and copper, plasma albumin and total protein were determined in the blood of 232 geriatric patients in long-stay wards, and in twenty-four age- and sex-matched persons, active and living in their homes. Intakes of dietary Zn, Cu, protein and fibre were also calculated for 126 of the patients and for the control group.

Mean intakes of the nutrients did not differ between the patient group and the controls, but in the patients, the mean concentrations of leucocyte Zn, serum Zn and Cu, and plasma albumin and total protein, as shown in the Table, were all significantly lower.

			Zn					
			Serum (μM)	Leucocyte (μg/g dry matter)		Serum Cu (μM)	Plasma albumin (g/l)	Plasma total protein (g/l)
	n							
In-patients	232	Mean	10·8***	63·3***	(*n* 218)	18·1**	37·6***	68·1*
		SE	0·1	0·8		0·2	0·2	0·4
		Range	5–19	37–98		2–39	26–45	54–88
Controls	24	Mean	12·7	72·8	(*n* 20)	20·0	42·0	70·6
		SE	0·3	1·9		0·6	0·6	0·9
		Range	10–16	62–97		15–25	35–48	65–80

Mean values were significantly different (Mann-Whitney U test): *$P<0.18$, **$P<0.01$, ***$P<0.001$.

Among the patients there was no significant correlation between Zn intake and either serum or leucocyte Zn, nor between serum Zn and Cu and leucocyte Zn. These indices and plasma albumin correlated poorly with intakes of the other nutrients. Plasma albumin and serum Zn correlated significantly (r 0·459, P = 0·001) and both correlated negatively with age (r −0·146, $P = 0.026$; r −0·150, P = 0·024) as did protein intake (r −0·187, $P = 0.036$): leucocyte Zn did not. Plasma total protein correlated significantly with serum Cu (r 0·183, $P = 0.007$) and the intake of protein (r 0·256, $P = 0.004$) but not of Zn or Cu.

Dietary intakes did not correlate significantly with the Isaacs Walkey score, used to assess mental clarity. The score correlated with plasma albumin (r 0·174, P = 0·001), total protein (r 0·153, $P = 0.020$) and serum Cu (r 0·142, $P = 0.038$), indicating significantly lower values in the demented patients, but not with serum or leucocyte Zn.

Hinks, L. J., Colmsee, M. & Delves, H. T. (1982). *Analyst* **107**, 815–823.

Dietary intake of zinc and copper in long-stay geriatric patients. By E. C. HENERY[1] and R. G. SMITH[2], [1]*Department of Nutrition and Dietetics and* [2]*Department of Geriatric Medicine, City Hospital, Edinburgh EH10 5SB,* and W. STAFFORD, *Department of Biochemistry, University of Edinburgh, Edinburgh EH8 9YL*

Dietary analysis was carried out in five continuing-care geriatric units in the Edinburgh area. Dietary intakes were calculated in 126 patients (mean age 82·6 years) and twenty-four age-matched controls (mean age 81·9 years). In one unit the menu was measured on three consecutive days to give a dietary analysis, and the controls and the remaining units gave detailed dietary accounts. Some of the diets for long-term patients were supplemented with food brought in by relatives or purchased by themselves; these included sweets, biscuits, fruit and squash. Values for these supplements are included in the results which can be related to the data on serum biochemistry given by Stafford *et al.* (1987).

Dietary intakes of patients and controls

Geriatric units	Protein (g/d)	Zinc (mmol/d)	Copper (mmol/d)	Fibre (g/d)
1†	75·0	0·145	0·035	16·7
2	56·4	0·114	0·015	8·8
3†	61·5	0·147	0·028	15·1
4	63·3	0·13	0·021	11·9
5†	65·7	0·149	0·022	19·5
Controls	64·0	0·113	0·019	18·0
RDA	47·0–60·0	0·15–0·23	0·035–0·05	30·0*

RDA, recommended daily amounts (Department of Health and Social Security, 1979).
*National Advisory Committee on Nutrition Education (1983).
†Received bran supplement.

The results show important differences in nutritional intakes between the various units. For example, the levels of Zn and Cu fell below the RDA in Unit 2 and the control group. The levels of protein varied, all being above the RDA, but they were significantly lower in Unit 2 than in the other units and in the controls.

In Units 1, 3 and 5 the diet was supplemented with milky drinks, e.g. 'build-up' and bran was added to the porridge. The results for fibre show that in Units 1, 3 and 5 levels were significantly higher than in those units not using bran. The control level of fibre (18 g/d) was within the normal range of 15–25 g/d taken in a Western diet. The interaction of Zn and fibre has been noted by Stamp (1978) who states that Zn absorption is affected by fibre (phytate) levels. It may, therefore, be disadvantageous for elderly patients to consume high-fibre foods as it may lead to trace-element deficiencies.

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Cobalt, copper and molybdenum in Scottish soils. By MICHAEL L. BERROW, *Department of Spectrochemistry, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ*

Cobalt, copper and molybdenum are of particular importance to animal health in Scotland and soil analysis is a useful aid in identifying areas where problems are likely to arise. The diagnostic criteria used for these three elements have been detailed in a recently published bulletin (Macaulay Institute for Soil Research/Scottish Agricultural Colleges, 1985). Analyses of some 1000 soil profiles, comprising over 4000 samples, has provided comprehensive information on the distribution of these elements in Scottish soils (see Table 1).

Table 1. *Co, Cu and Mo in Scottish soils* (μg/g)

	Total contents				Extractable contents	
	Range	Mean	Normal range	Derived mean	Normal range	Derived mean
Co	0·4 – 400	12·7	0·84– 62	7·6	0·05–4·5	0·3
Cu	<1·0 –2500	18·0	0·93–110	10·0	0·08–9·8	0·86
Mo	0·05– 40	1·5	0·21–4·9	1·0	–	–

Soils derived from granite or fluvioglacial sands and gravels have low contents of both Co and Cu and deficiencies of these elements are found on these soil types. Soils derived from granite, mixed igneous and metamorphic rocks, Caithness flagstones and argillaceous schists sometimes contain large amounts of Mo. The high-Mo soils occur within localized areas on these parent materials and are probably related to mineralization. There are several hundred farms in northern Scotland, principally in Aberdeenshire, Easter Ross and Caithness, where excessive Mo causes problems in animal health.

All three elements are actively mobilized by naturally poor soil drainage conditions which increase their availability for plant uptake (Berrow *et al.* 1983). The mobilization of Co and Cu by waterlogging has also been illustrated by Berrow & Ure (1986).

Recent evidence from field-plot studies has shown that most herbages grown on freely drained soils contain 0·08 μg Co/g dry matter or less and are thus nominally deficient in Co for the health of ruminants. In the cases of Cu and Mo, the availability of both elements is increased in poorly drained soils, but while the mobilized Cu is taken up into the leaves to a very limited extent, Mo is taken up much more readily. Poor drainage thus tends to decrease the Cu:Mo ratio in herbage and problems of Mo excess such as 'teart' or 'black scour' are likely to be more prevalent on poorly than on comparable freely drained soils.

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Berrow, M. L. & Ure, A. M. (1986). *Environmental Geochemistry and Health* **8**, 19–24.

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Selenium pathways in the soil–plant–animal system. By SHEILA VAN DORST and IAIN THORNTON, *Applied Geochemistry Research Group, Imperial College, London,* and WANG WUYI, *Institute of Geography, Academia Sinica, Beijing, China*

An investigation into the flux of selenium from the environment to grazing sheep is reported. The transfer of Se from the environment to livestock can be divided into four major pathways: air, water, soil (ingestion) and plant. North Wales, a low–marginal Se area of Britain, and a Se sufficient area, West Derbyshire, were studied. Rock, soil, water, herbage, wool and blood samples were collected on a seasonal basis from fourteen farms. A high correlation (r 0·875) was found between blood glutathione peroxidase (*EC* 1.11.1.9; GSHPx) activity and wool Se concentrations. Wool was therefore used as a measure of the Se status of the sheep and also represented the final terminal through the transfer procedure.

Table 1 indicates differences in Se concentration between samples from the study areas. Se levels in air were not monitored.

Table 1. *Concentration of Se* (μg/g), *and GSHPx activity*

	Rock		Soil		Herbage		Wool		GSHPx activity	
Location	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
North Wales	0·08	0·042	0·47	0·095	0·03	0·003	0·07	0·010	49	19·8
West Derbyshire	1·32	0·213	2·00	0·213	0·22	0·107	0·25	0·101	189	23·9

Seasonal changes in soil, herbage and wool were also observed. Se concentrations for wool and herbage were found to peak in March. Taking seasonal changes into account and making assumptions concerning the consumption and availability of water, soil and herbage by sheep, estimates for the transfer of Se from the environment to the animal can be made. Table 2 gives estimations of the flux of Se from the environment to sheep seasonally. Differences in daily Se intake between areas can be observed.

Table 2. *Total intake of Se by sheep* (μg/d)

	October	March	June	September
North Wales	24·4	48·1	13·2	11·8
West Derbyshire	152·1	204·1	114·7	91·1

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Assessing the availability of ingested soil cobalt for the synthesis of vitamin B_{12} in the ovine rumen. By JOCELYN BREBNER and N. F. SUTTLE, *Moredun Research Institute, Edinburgh EH17 7JH* and I. THORNTON, *Royal School of Mines, London SW7 2BP*

Soils contain far more cobalt than the pasture they support. The continuous ingestion of soil as a pasture contaminant may, therefore, enhance vitamin B_{12} synthesis and prejudice the prediction of Co deficiencies in grazing animals from herbage analyses. The capacity of a range of soils, differing in total (T) and acetate-extractable (AE) Co concentration, to supply Co for cobalamin (cbl) synthesis was, therefore, investigated.

In three experiments, groups of four or five Scottish Blackface ewes were given 0·7–0·8 kg/d of a pelleted diet (after Suttle & Field, 1968), low in Co (around 0·03 mg/kg dry matter (DM)) and supplemented with 17·5, 35·0 or 70 g various soils/kg for 21 d (see Table). In each experiment one group received no soil and between experiments the low-Co diet was given so that sheep began Expts 2 and 3 in a Co-depleted state. The cbl concentrations in strained rumen fluid obtained by stomach tube were measured by the method of McDonald & Suttle (1986).

Expt no.	Soil	Level of soil intake (g/kg DM per d)	Co intake from soil (μg/d)		cbl in rumen fluid (nmol/g DM)					
					Day 0		Day 21		Day 28	
			T	AE	Mean	SE	Mean	SE	Mean	SE
1	–	0	–	–	1·20*	–	1·12	0·17	0·51	0·08
	A	17·5	151	18·6			1·53		0·58	
	A	35·0	301	37·2			1·67		0·89	
	A	70·0	602	74·2			1·94		0·63	
2	–	0	–	–	0·42	0·07	0·48	0·10	0·37	0·08
	B	35·0	255	39·9	0·16		0·84		0·53	
	B	70·0	510	79·9	0·25		0·86		0·34	
	C	35·0	240	27·0	0·33		0·93		0·60	
	C	70·0	480	53·9	0·21		1·12		0·57	
3	–	0	–	–	0·49	0·08	0·34	0·05	–	
	D	35·0	190	11·4	0·41		0·45		–	
	E	35·0	336	27·4	0·28		0·53		–	
	F	35·0	157	9·0	0·32		0·55		–	

*Extrapolated value.

The results in the Table show increases of 0·04 to 0·91 nmol/kg DM in rumen cbl concentrations after ingesting soil for 21 d. In Expt 2, responses were rapid with near maximum values recorded after only 7 d soil ingestion: depletion was equally fast. Rumen cbl did not increase in proportion to soil intake in Expts 1 and 2. At the 35 g/kg level of soil addition (Expts 2 and 3), increases in rumen cbl from 0 to 21 d were related to AECo rather than TCo with rumen cbl = 0·00178 AECo + 0·0745 (r 0·89; 5 df). The vitamin B_{12} status of grazing animals might be more closely related to the AECo concentration in the soil than Co concentration in a carefully washed herbage sample.

McDonald, P. M. & Suttle, N. F. (1986). *British Journal of Nutrition* **56**, 369–378.
Suttle, N. F. & Field, A. C. (1968). *Journal of Comparative Pathology* **78**, 351–362.

Microbicidal activity of neutrophils of cobalt-deficient and repleted calves. By JESSIE E. PATERSON and A. MACPHERSON, *West of Scotland Agricultural College, Auchincruive, Ayr KA6 5HW*

Previous studies (MacPherson *et al.* 1976) have demonstrated enhanced susceptibility to infection in cobalt-deficient sheep and impairment of immunity in calves (Wright *et al.* 1982). Similar effects have been reported by Boyne & Arthur (1981) and Jones & Suttle (1981) when diets deficient in either selenium or copper were given to calves and sheep respectively. More recently, MacPherson *et al.* (1987) have recorded some impairment of the immune function in vitamin B_{12}-deficient cattle infected with intestinal parasites, and Fisher & MacPherson (1986) have shown similar effects in Co-deficient ewes with a consequent marked decrease in the viability of their lambs.

The present study was designed to examine the effect of maintaining calves on a Co-deficient diet on their immune status as assessed by the neutrophil function test (NFT) where the ability of the neutrophils to kill the yeast *Candida albicans* is measured. In addition, the effects of Co repletion on the NFT were monitored. Co status was assessed by vitamin B_{12} assay and also by measuring serum methylmalonic acid concentrations in order to assess its potential as a diagnostic marker of clinical or sub-clinical Co deficiency.

Six Friesian calves were fed on a Co-deficient ration of hay and concentrates (<0·04 mg Co/kg dry matter). Vitamin B_{12} values, which were initially just within the normal range (>200 ng/l), fell to 150 ng/l within 9 weeks and to around 100 ng/l within 13 weeks on experiment. They remained at this level with only minor fluctuations until week 36 when levels fell to 50 ng/l. The results of the NFT have followed a similar pattern with the percentage kill falling from an initial value of 35% to 15% by 18 weeks on experiment and to 7% by week 41. Methylmalonic acid concentrations increased from 1·5 μmol/l to around 3·0 μmol/l after 36 weeks. Early indications are that while Co repletion increased vitamin B_{12} values after 5 weeks, microbicidal activity only showed a gradual increase from 14 to 17% over that period.

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Copper supplementation during pregnancy can reduce perinatal mortality and improve early growth in lambs. By N. F. SUTTLE and D. G. JONES, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH,* and J. A. WOOLLIAMS and C. WOOLLIAMS, *AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS*

Lambs of breeds vulnerable to copper deficiency grazing recently limed and reseeded pastures can suffer increased incidence of fatal infections and retarded growth with delayed marketing of survivors. Although Cu supplementation of lambs at 6 weeks of age improved survival and growth, losses by this time were already noticeable. We have therefore examined the effects of treating the ewe orally with 4 g cupric oxide needles (May & Baker Ltd., Dagenham, Essex) 8 weeks before lambing started.

In a flock of 299 ewes, comprising Scottish Blackface (B, *n* 58), Welsh Mountain (W, *n* 58) and lines from a B × W cross, selected for low (L, *n* 89) or high (H, *n* 94) plasma Cu, roughly half of each breed type were treated with Cu. The ewes were divided into early and late sets (1 and 2) for lambing when the pastures grazed contained (mmol/kg dry matter) 0·085 and 0·043 Cu, 0·026 and 0·040 molybdenum, 111 and 134 sulphur respectively. The number of lambs born to unsupplemented and supplemented ewes respectively were: B, 51 and 48; W, 35 and 47; L, 72 and 79; H, 71 and 77. The probabilities of lambs dying in the first 4 weeks of life and the live weight of survivors at 6 weeks are shown in the Table.

	Cu supplement	Set	Line		Breed	
			L	H	B	W
Mortality at 4 weeks (as proportion of lambs born)	Without	1	0·253	0·120	0·247	0·046
	Without	2	0·344	0·174	0·337	0·070
	With	1	0·039	0·115	0·104	0·017
	With	2	0·059	0·167	0·152	0·026
Live weight at 6 weeks* (kg)	Without	1	12·3	12·4	15·4	12·9
	Without	2	14·1	15·2	17·4	13·9
	With	1	12·4	13·2	15·9	13·0
	With	2	15·0	16·8	18·7	14·8

*Approximate SE: 0·47 for L and H sub-classes, 0·63 for B and W sub-classes.

The Cu supplement reduced mortality most in breed groups known to be susceptible to Cu deficiency (L and B), the total losses in these groups falling from 34/123 to 9/127. Only seven of the casualties amongst unsupplemented lambs had swayback. Mortality was correlated between sets and breed groups with plasma Cu and erythrocyte superoxide dismutase (*EC* 1.15.1.1) at the first sampling of surviving lambs when they were 6 weeks old: by this time a significant growth improvement was evident in Cu-treated survivors ($P<0·001$). Thus improved survival and early growth can be obtained by supplementing the Cu-deficient ewe during pregnancy.

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The effect of molybdate compounds on the biliary excretion of copper by sheep. By Y. KE and H. W. SYMONDS, *Department of Animal Physiology and Nutrition, University of Leeds, Leeds LS2 9JT*

Intravenous injection of tetrathiomolybdate (TTM) decreases the copper content of the liver in sheep (Gooneratne *et al.* 1981). Part of this decrease may be due to an increased output into bile. To study this aspect the effect of a single injection of molybdate or TTM on the biliary excretion of Cu was measured.

Four castrated male sheep were surgically prepared to enable bile to be collected. They were given 1 kg grass nuts/d providing 9·4 mg Cu. For 15 d before injection of the molybdate compounds, the sheep were given a daily oral dose of 20 mg Cu as $CuCl_2$. Each sheep was given in turn, intravenously, one of the following treatments (allowing at least 3 d between different treatments): no injection (control); 21·4 mg MoO_4 as ammonium molybdate; 50–175 mg MoS_4 as ammonium TTM in 25 mg increments. Bile flow rate was measured by weighing the volume secreted during consecutive 30 min periods. Samples (4 ml) were taken for analysis and replaced by a similar volume of pooled bile to prevent depletion of bile salts. On each treatment day, flow was measured for 10 h starting at 09·00 hours. The injections of the molybdate and TTM were given between 09·20 and 09·25 hours. To determine the time during which the compounds acted, samples were taken from some sheep at 16, 19, 21, 24, 27 and 30 h after dosing.

The Table shows the total quantities of Cu excreted in bile by each sheep during the first 10 h after dosing with TTM in experimental (E) and control (C) periods.

	Total Cu excreted in bile in 10 h (μg)							
Sheep no. . . .	1		2		3		4	
TTM (mg)	C	E	C	E	C	E	C	E
50	193	653	132	445	63	351	85	445
75	196	752	135	588	61	422	83	564
100	185	894	127	847	60	767	83	685
125	186	1176	129	947	63	825	84	828
150	194	1536	132	980	62	853	84	846
175	196	1586	137	985	60	862	84	848

Molybdate produced a 34–46% increase in Cu excretion in three sheep only. TTM produced a much greater increase in Cu excretion. The effect was dose related up to a maximum at 125 mg TTM in three sheep and 150 mg TTM in one sheep. The relation between the intravenous dose of TTM and total Cu excreted up to the maximum was described by $Y=122+6{\cdot}6\,X$ (r 0·9705) where Y is μg Cu and X is mg TTM injected. Cu excretion had returned to normal values by 30 h after dosing.

Gooneratne, S. R., Howell, J. McC. & Gawthorne, J. M. (1981). *British Journal of Nutrition* **46**, 457–467.

Effect of endogenous status of zinc on copper absorption and its application to the treatment of Wilson's disease. By ZAFRALLAH T. COSSACK and CORNELIS J. A. VAN DEN HAMER, *Department of Nuclear Biotechnique, Interuniversity Reactor Institute, Mekelweg* 15, 2629 *JB, The Netherlands*

Zinc, in therapeutic dosages, has been shown to inhibit copper absorption in patients with Wilson's disease. Brewer *et al.* (1983) observed that in patients with this disease, 3–4 weeks of Zn supplementation is necessary to achieve zero or negative Cu balance. In addition, van den Hamer *et al.* (1984) observed that the adverse effect of Zn on Cu absorption, in Wilson's patients and controls, continued for 12–24 d following the withdrawal of Zn therapy. The present study was conducted to substantiate these observations, using an experimental model.

A group of mice was given 30 μg Zn/kg for a period of 70 d (control) and a second group was given 1000 μg Zn/kg for the first 35 d (repletion) after which they were switched to the basal diet (6 μg Zn/kg) for the following 35 d (depletion). Whole-body retention (WBR) of ^{64}Cu was measured at intervals throughout the experimental period. Endogenous or metabolic faecal Zn (MFZn) was determined at the end of repletion (zero time) and at intervals during depletion.

Results from this experiment indicated that the effect of Zn on ^{64}Cu absorption continued for up to 21–35 d following the withdrawal of Zn supplement. In addition, WBR of ^{64}Cu correlated significantly (r −0·92, P<0·0001) with MFZn.

We conclude that: (1) endogenous Zn secreted into the gastrointestinal tract plays a major role in inhibiting Cu absorption, thus the maintenance of high endogenous Zn status is essential for maximal inhibition of Cu absorption; (2) ^{64}Cu-loading test should not be used in checking for compliance with Zn therapy in patients with Wilson's disease.

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Copper retention in the human liver: a histochemical and immunocytochemical study. By J. P. CLARKSON, M. E. ELMES and B. JASANI, *Department of Pathology, University of Wales, College of Medicine, Cardiff CF4 4XN*

The bile is an important excretory pathway for copper and if functional or obstructive lesions to normal bile flow are present, hepatic Cu accumulation occurs. Other conditions leading to an increased liver Cu are genetic diseases such as Wilson's disease in which there is a failure to incorporate Cu into caeruloplasmin and to excrete Cu in the bile, and environmental toxicoses of which Indian childhood cirrhosis is probably an example.

The current methods of diagnosing hepatic Cu accumulation include direct analysis of liver tissue using atomic absorption spectrophotometry and neutron activation analysis, in which a biopsy sample is usually destroyed, and histochemical stains for Cu and Cu-associated protein (CAP) which can be performed on histological sections of routine biopsies. Stains for Cu may be unhelpful in Wilson's disease as they can be negative in a tissue which, on biochemical analysis, contains large amounts of Cu.

The metal-binding protein metallothionein (MT) is present in liver and we have developed a sensitive immunocytochemical technique to demonstrate immunoreactive MT in human liver in routinely formalin-fixed, wax-embedded histology sections. The following were studied histologically: normal liver biopsies (n 6), extrahepatic biliary obstruction (n 5), cirrhosis (n 3), cholangitis (n 3), Indian childhood cirrhosis (n 16), primary biliary cirrhosis (n 2), Wilson's disease (n 5). In all cases the sections were examined for the presence of MT and in the majority this was correlated with histochemically detectable Cu, using rubeanic acid, and CAP, using orcein.

Normal adult liver contained no stainable Cu or CAP and five contained small amounts of cytoplasmic MT. Indian childhood cirrhosis showed Cu and CAP in coarse cytoplasmic granules in paraportal regions, and MT in a diffuse cytoplasmic distribution that did not correspond with Cu and CAP. In primary biliary cirrhosis large amounts of diffuse cytoplasmic MT were found. In Wilson's disease bizarre hepatocytes containing large amounts of diffuse MT were demonstrated throughout the tissue whereas Cu and CAP were largely found in granules in paraportal regions. In one case penicillamine treatment resulted in a marked decrease in MT.

We conclude that conventional histochemical stains for Cu only demonstrate large granules which are probably lysosomes and that cytoplasmic Cu, which is not shown by this technique, may be bound to the large amounts of MT demonstrated in conditions of Cu retention.

Intracellular events in the liver and kidney of copper-loaded rats in relation to Cu-storage diseases in man and animals. By S. HAYWOOD, R. M. BATT and M. LOUGHRAN, *Department of Veterinary Pathology, University of Liverpool, PO Box 147, Liverpool L69 3BX*

Hereditary aberrations of copper metabolism in which accumulation of the metal occurs in the liver and kidneys of man and animals, with pathological consequences, are well recognized. Wilson's disease (WD) was established as an autosomal recessive Cu-storage disease in man (Bearn, 1953). In dogs, Bedlington terrier toxicosis has been found to be similarly inherited (Johnson *et al.* 1980). More recently, a Cu-associated hepatotoxicosis has been reported in the West Highland White breed (Thornberg *et al.* 1986). It is not clear why Cu accumulates and how excess Cu damages the liver and other tissues. The present study was designed to investigate the subcellular localization of Cu during Cu loading and its consequences.

Male Wistar rats, of uniform age and weight, were fed on a powdered diet (Labsur animal diet, RHM Agriculture South Ltd) with a Cu content of 3 g/kg for 14 weeks and killed sequentially during this period. Cu in liver and kidney cortex was identified histochemically and assayed in homogenates and gradient fractions following analytical subcellular fractionation on reorientating sucrose density gradients (Haywood *et al.* 1985).

Cu was localized initially in hepatic lysosomes. Rapid accumulation of Cu then occurred in hepatic and renal fractions, which coincided with tissue necrosis. It also accumulated in the cytosol of the cells of the proximal renal tubules from which subsequently it appeared to be excreted, in parallel with declining hepatic Cu levels.

The results suggest that cellular injury was consequent on nuclear destabilization rather than lysosomal disruption and that Cu tolerance was associated with a redistribution of Cu and the renal excretion of excess. A similar situation may occur in WD in which very high Cu values occur in young patients, whereas the later stages of the disease are characterized by a somewhat lower hepatic Cu load with marked urinary excretion (Sternlieb & Scheinberg, 1968). Parallel changes in the Cu load have been reported in Bedlington terriers (Twedt *et al.* 1979). However, in contrast to the rat, the overall hepatic Cu concentrations remain high in these diseases and the injury progressive. Thus, susceptibility to Cu toxicity may in part be due to a failure of intracellular homeostasis concerned with the distribution, binding and subsequent excretion of Cu.

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Nitrogen losses from the human small bowel: obligatory losses and the effect of diet. By A. CHACKO and J. H. CUMMINGS, *MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL*

Significant protein breakdown may occur in the human hind-gut (McFarlane *et al.* 1986), the principal substrate for this being material entering from the ileum. Although there are reports of total nitrogen losses in ileostomy effluent of 1–2 g/d, little is known of the relative contributions from diet and endogenous sources or of the form in which N enters the large bowel. In order to determine N losses from the small bowel we gave, in random order, three diets for 2 d each to six patients with ileostomies, all of whom had colectomy for ulcerative colitis and were without any small bowel disease. Endogenous losses were measured when the subjects were on a low-N diet (LND; 0·17 g N/d). The effect of the physical form of food was tested by feeding the LND + 100 g cooked soya beans, either whole or puréed (5·9 g N/d). The effect of dietary fibre was assessed by feeding a high-fibre diet (10·6 g N/d; 40 g non-starch polysaccharides/d). N loss from the small bowel and its composition were determined by measuring total N, protein, free amino acids, urea and ammonia in the ileal effluent.

Despite the low protein intake, exocrine pancreatic function was normal on LND (effluent amylase 223 (SE 48) units/ml; total proteolytic activity $4·6 \times 10^4$ (SE 0·7) μg azocasein hydrolysed/h per ml). The Table shows obligatory N losses and the effect of diet. N loss with whole soya beans was significantly higher than that with puréed beans ($P<0·001$). N losses on the high-fibre diet did not differ significantly from that of ten out-patients with ileostomies who, on a dietary fibre intake of 15 g/d, collected 24 h effluent which contained 2·1 (SE 0·12) g N/d.

	LND			LND + whole soya beans			LND + puréed soya beans			High-fibre diet		
	Mean	SEM		Mean	SEM		Mean	SEM		Mean	SEM	
Total N loss (g/d)	0·91	0·04		2·26	0·15		1·42	0·12		2·17	0·17	
N absorbed (%)	–	–		73·0	3·3		90·1	1·3		88·5	0·7	
Composition of N loss:												
Protein	0·43	0·03	(47%)	1·16	0·13	(51%)	0·68	0·06	(48%)	0·99	0·08	(45%)
Amino acid	0·05			0·26			0·16			0·20		
Urea	0·04			0·06			0·05			0·08		
Ammonia	0·01			0·01			0·02			0·03		
Peptide (by difference)	0·38			0·77			0·51			0·87		

We conclude that obligatory N losses from the small bowel were about 1 g/d. The physical form of food influenced N loss but dietary fibre content did not. N loss was mainly in the form of protein and peptides.

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The metabolism of a high intake of nicotinamide in choline-deficient rats.

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One rationale for the use of very high intakes of nicotinamide in the treatment of schizophrenia is that metabolism to *N*-methyl nicotinamide will deplete tissue pools of methyl donors, and hence reduce the formation of (possibly psychotogenic) methylated metabolites of neurotransmitters. Depletion of methyl donors would also reduce the ratio of phosphatidyl choline (PC):phosphatidyl ethanolamine (PE) in membrane phospholipids.

Five rats per group were maintained for 7 weeks from weaning on a maize–gelatine–sucrose diet (Bender *et al.* 1982) providing 1500 mg nicotinamide (the recommended amount for the rat is 15 mg) and 0·9 mg tryptophan/kg. The deficient diet contained no choline (1·8 g/kg in the control diet) and no added methionine in the amino acid mixture (control 2·25 g/kg).

Urinary excretion of nicotinamide metabolites (mmol/mol nicotinamide consumed) determined by high-pressure liquid chromatography

Group . . .	Control		Choline deficient	
	Mean	SD	Mean	SD
Nicotinamide	54	13·5	43	8·4
Nicotinic acid	4·5	0·49	5·7	0·68
N-Methyl nicotinamide	20	3·2	13	2·3
Pyridone	26	5·0	5·7	0·99
Nicotinamide *N*-oxide	21	3·5	36	4·4
Nicotinuric acid	5·6	0·72	9·1	0·84

There were no significant differences in the ratio of PC:PE in the phospholipid fractions isolated.

	PC:PE				
Group . . .	Brain	Liver	Pancreas	Heart	Blood
Control	1·1	1·6	1·9	1·2	1·4
Choline deficient	1·1	1·4	1·8	1·3	1·6

Although choline deficiency reduced the excretion of methylated metabolites of nicotinamide, and increased non-methylated metabolites, these results show no evidence of 'methyl depletion' induced by this very high intake of nicotinamide.

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Bender, D. A., Magboul, B. I. & Wynick, D. (1982). *British Journal of Nutrition* **48**, 119–127.

Comparison of Novomine, a locally manufactured amino acid solution, with commercially available solutions, in a District General Hospital. By M. B. CLAGUE, P. D. WRIGHT, G. DALE, P. MCKENZIE, P. HOPLEY and M. EARNSHAW, *Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE*

Parenteral nutrition is now an accepted modality of nutritional support for patients unable to tolerate oral nutrition. However, such treatment is expensive, the major component of cost in non-lipid regimens being the amino acid solution. The presence of a District Manufacturing Unit within the Pharmaceutical Department facilitated the production of a locally formulated amino acid solution (Novomine) and comparison was made in its clinical use with commercially available solutions.

A total of forty-eight patients referred for standard parenteral feeding (13–14 g nitrogen, 8·4 MJ as glucose, electrolytes, trace elements and vitamins in approximately 2·5 litres) were randomly allocated to receive differing N sources (Synthamin 14, Freamine II, Freamine III or Novomine). Solutions were coded and supplied blind to the clinician. Clinical and biochemical monitoring was undertaken on each patient and plasma aminograms were measured on days 0, 1, 3 and 7 in a number of patients.

	Synthamin 14		Freamine II*		Freamine III		Novomine	
	Median	Range	Median	Range	Median	Range	Median	Range
No. of patients	14		4		12		18	
Period of experiment (d)	10	3–46	5	4–28	7	4–23	9	2–38
No. of clinical events	11		2		9		16	
Metabolic acidosis (d)	1(14)		0		1(4)		2(1, 2)	
Elevated alkaline phosphatase (*EC* 3.1.3.1) i.e. >130 i.u./l	6		1		6		6	
Sepsis	6		1		7		9	
Mortality	3		0		2		5	

*Freamine II withdrawn commercially after commencement of trial.

No differences attributable to the N source were identified in this group of ill patients, although the early development of a metabolic acidosis in two patients receiving Novomine warrants further investigation. Plasma aminograms all showed qualitatively similar patterns with some quantitative differences related to formulations.

This small study illustrates that an amino acid solution produced in District Pharmaceutical Manufacturing Unit produces similar results to commercially available products. It can, however, reduce costs and could be flexible enough to allow formula modifications. Analysis of data from a larger study is awaited.

Relation between osmolality of the diet and diarrhoea in enteral feeding.

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Although enteral nutritional support is widely used, troublesome complications may occur. Recent research has concentrated on the aetiology of diarrhoea and there has been much debate on whether its prevalence is related to the osmolality, constituents of the enteral regimen or concomitant antibiotic therapy.

To examine the relation between osmolality and diarrhoea, surgical patients were randomized to receive one of three whole-protein feeding regimens: group 1, 450 mosmol/kg; group 2, 300 mosmol/kg; and group 3, 300 mosmol/kg (medium chain triglyceride based). All feeds were prepared aseptically and administered by 24 h nasogastric infusion. Tolerance, actual intake and antibiotic therapy were recorded prospectively and diarrhoea documented by a stool scoring system (firm = 1 point, soft = 2, liquid = 3).

Groups were well matched for sex, age, weight and diagnosis. Forty-nine patients received post-operative nutritional support and nine patients were fed pre-operatively or had no surgical intervention.

Group . . .	1 (*n* 23)		2 (*n* 17)		3 (*n* 18)	
	Mean	SE	Mean	SE	Mean	SE
Age (years)	66	12	61	12	62	10
Period fed (d)	10	4	9	3	10	5
Energy intake/d:						
MJ	7·9	1·9	8·5	1·5	7·7	1·5
kcal	1894	457	2020	348	1828	366
Nitrogen intake/d	10·7	2·6	12·9	2·2	10·5	2·1
Stool frequency/d	0·6		0·4		0·4	
Average stool score	1·9		1·8		1·4	

The incidence of tube blockage, abdominal distention, nausea and vomiting was similar in each group. Episodes of diarrhoea occurred more frequently in group 1 (eight patients) than in either groups 2 or 3 (one patient each, $P<0·05$; chi squared). Diarrhoea was associated with antibiotic therapy, except in three patients in group 1. Constipation occurred in six patients in group 1, nine patients in group 2 and eight patients in group 3.

The results suggest that both osmolality and antibiotic therapy may influence the tolerance of enteral feeding regimens in surgical patients.

Liver function test abnormalities during enteral nutrition. By R. A. RICHARDSON[1], A. SHENKIN[2] and O. J. GARDEN[1], *Surgical Nutritional Advisory Group, University [1]Department of Surgery and [2]Department of Biochemistry, Royal Infirmary, Glasgow G31 2ER*

Liver function test abnormalities are common in patients receiving artificial nutrition, especially intravenous nutrition. The reason is not clear, although they are often associated with sepsis (Robertson *et al.* 1986). There have also been reports of liver enzyme abnormalities among enterally fed patients. The aim of the present study was to determine the pattern of liver function tests in patients on enteral nutrition support receiving a minimum of 6·3 MJ (1500 kcal)/d for at least 2 weeks. In addition, factors which might be responsible for such changes were defined. Patients with normal values for serum bilirubin (bil), aspartate aminotransferase (*EC* 2.6.1.1; AST), alanine aminotransferase (*EC* 2.6.1.21; ALT), alkaline phosphatase (*EC* 3.1.3.1; AP) and γ-glutamyl transpeptidase (*EC* 2.3.2.2; γ-GT) at the start of the study period had these liver function tests repeated weekly during nutritional support. Body-weight, arm muscle circumference and serum albumin were measured on entry to the study.

Nineteen patients (thirteen male, six female) with a mean age of 63 (range 41–81) years fulfilled the entry criteria. Eleven patients were fed post-operatively while eight were fed pre-operatively or had no surgical intervention. The mean feeding period was 30 (range 14–68) d with a daily intake (mean and SE) of 8·7 (1·5) (range 6·3–11·7) MJ (2090 (358) (range 1500–2795) kcal) and 11·5 (2·0) (range 7·7–15·3) g nitrogen.

Eighteen patients were given 'whole-protein' diets while one patient received a peptide diet. Eleven patients (group 1) developed abnormal liver function tests (10 γ-GT, 8 AP, 6 AST, ALT, 0 bil) while eight patients remained normal (group 2). One patient from each group received a blood transfusion. There was no significant difference between the two groups at the start of enteral feeding with respect to body-weight (group 1: 46·2 (7·5) kg; group 2: 46·2 (10·0) kg), percentage arm muscle circumference (81·9 (9·1); 82·2 (13·3)) or serum albumin (29·6 (6·1) g/l; 31·8 (6·0) g/l). Six of the eleven patients in group 1 developed septic complications, three wound infections and three chest infections. No patient in group 2 had clinical or bacteriological evidence of infection ($P<0·01$; Fisher's exact test) during nutritional support.

It is concluded that γ-GT is the most sensitive biochemical indicator of liver dysfunction during enteral nutrition. Abnormalities in liver function tests are more likely to be due to associated clinical complications than the enteral nutritional support alone.

Robertson, J. F. R., Garden, O. J. & Shenkin, A. (1986). *Journal of Parenteral and Enteral Nutrition* **10**, 172–176.

Mechanisms contributing to the rachitogenic properties of maize. By M. R. SLY, D. B. DU BRUYN, D. J. ROBBINS, W. H. VAN DER WALT and J. N. VAN DER MERWE, *National Food Research Institute, CSIR, PO Box* 395, *Pretoria* 0001, *South Africa*

When maize is consumed in a vitamin-D-deficient diet, it increases both the speed of development and degree of severity of rickets and osteomalacia in young baboons (Sly *et al.* 1984). Because this cereal is such an important dietary staple, attempts were made to obtain a better understanding of the mechanisms by which it produces its deleterious effects. Possibilities include enhancement of vitamin D, phosphorus or calcium deficiency by maize through its fibre or phytate components or both.

Four groups of four or five baboons were placed on diets free of vitamin D but containing 3 g Ca/kg and the following amounts of 'available' P (from casein, added inorganic P or non-phytate maize P) and phytate P (from maize or sodium phytate):

	P (mg/kg diet)			
Group . . .	1	2	3	4
Available P: Casein	900	900	900	900
Maize	350	350	–	–
Inorganic salts	–	1060	350	350
Phytate P: Maize	1060	1060	–	–
Sodium phytate	–	–	–	1060

Within a few weeks, serum alkaline phosphatase (*EC* 3.1.3.1) levels had risen threefold, and serum inorganic P and 25-hydroxyvitamin D concentrations dropped to less than one-third of their initial concentrations. No group differences in the changes of these indices of rickets and osteomalacia were noted. Based on these serum values, maize does not appear to affect vitamin D status or the rate at which rickets and osteomalacia develops (those diets which were free of maize were, however, low in 'available' P).

In subsequent studies of short duration (2 weeks) the effects of maize fibre on the absorption of Ca and P by vitamin-D-replete animals were examined. Dietary and faecal mineral contents were expressed on a faecal marker basis, and absorption was calculated from plots of intake –faecal minerals *v.* intake for different, sub-optimal intakes of the mineral in question. Respective percentage absorptions (mean and SEM) of P and Ca were respectively 59·0 (9·5) and 90·2 (1·8) from a semi-synthetic diet, and 15·1 (9·2) and 65·7 (4·5) from a similar diet containing 150 g maize bran/kg. Tentative conclusions are that effects on P and perhaps Ca, rather than vitamin D metabolism, may be significant mechanisms contributing to the rachitogenic properties of maize.

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