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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Fifty-sixth Meeting of the Nutrition Society (One Hundred and Fortieth of the Scottish Group) was held in the School of Agriculture, 581 King Street, Aberdeen on Friday, 6 March 1981 when the following papers were read:

The effects of iron and molybdenum on copper metabolism in cattle. By W. R. HUMPHRIES, B. W. YOUNG, M. PHILLIPPO and I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Increased intakes of iron, equivalent to 1500–2000 mg Fe/kg diet, were found to decrease liver copper concentrations in cattle (Standish *et al.* 1971; Campbell *et al.* 1975). It is not clear, however, how far these results were influenced by the reduced food intake of the calves or by the concomitant increase in S intake (from the FeSO₄ supplement). The present experiment was undertaken therefore to determine whether a lower concentration of Fe, when included in a diet of suboptimal Cu content, was also capable of inducing Cu deficiency. In addition, since Fe and molybdenum frequently interact in biological systems, the effect of Mo supplementation was also examined.

Sixteen Hereford-Friesian heifers (100–180 kg liveweight) were allocated on the basis of weight and liver Cu content, to four groups, and were given a barley–straw ration *ad lib.* for 8 weeks. The diet contained about 4 mg Cu 2.8 g S and 250 mg Fe/kg DM and was supplemented with 0 or 800 mg Fe (as saccharated ferrous carbonate) and 0 or 5 mg Mo (as ammonium molybdate)/kg. The plasma and liver Cu and Fe concentrations were measured at 2–4 week intervals.

Time (weeks)	Control		Fe		Mo		Fe+Mo		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
0	106	12	95	16	108	17	112	19	
Liver Cu (mg/kg DM)	4	91	17	27	3	51	6	48	12
8	90	23	20	4	30	4	20	5	

There were no differences between the four groups in their mean daily live-weight gain or in their food intakes. Liver Cu concentrations were significantly reduced by about 70% within 4 weeks in the calves receiving the Fe supplement alone and were reduced in all Fe- and Mo-supplemented groups within 8 weeks (see Table). Plasma Cu gradually declined during the experiment and was significantly less in all the Fe- and Mo-supplemented calves than in the control calves after 4 weeks. Mean liver Fe concentrations at 8 weeks were 181, 208, 505 and 647 mg/kg DM in the control, Mo, Fe and Mo+Fe groups respectively and plasma Fe concentrations followed a similar pattern. The degree of transferrin saturation increased from about 40% in the control and Mo calves to 60 and 75% in the calves in the Fe and Mo+Fe groups respectively, comparable to the saturation found in cases of Fe overload.

The above findings confirm therefore that moderately increased Fe intakes in cattle rapidly reduce liver and plasma Cu concentrations to levels indicative of Cu deficiency. Indeed the decrease in Cu status induced by 800 mg Fe/kg DM was as great as that caused by 5 mg Mo/kg. Since in our experience silages in north-east Scotland frequently contain up to 2500 mg Fe/kg DM, probably as a soil contaminant, more attention should perhaps be directed to the inhibitory effect of Fe on Cu utilization.

Campbell, A. G., Coup, M. R., Bishop, W. A. & Wright, D. E. (1975). *Proc. N.Z. Soc. Anim. Prod.* **35**, 175.

Standish, J. F., Ammerman, C. B., Palmer, A. Z. & Simpson, C. F. (1971). *J. Anim. Sci.* **33**, 171.

Effect of variation in dietary iron concentration on copper metabolism in rats. By I. BREMNER and B. W. YOUNG, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It was shown that an increased dietary intake of iron by calves receiving a ration with suboptimal copper content resulted in a rapid decrease in liver and plasma Cu (Humphries *et al.* 1981). Moreover the combination of low Cu and high Fe intake caused major increases in plasma and liver Fe to levels indicative of Fe overload. In this investigation the effects of dietary supplementation with 50, 500 or 1000 mg Fe (as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)/kg diet were studied in thirty-six male rats. The rats received a semi-synthetic diet with either <1 or 3 mg Cu/kg and were killed after 11 weeks. Growth and food intake were significantly reduced in rats receiving the low-Cu diet but were not influenced by the Fe intake. Cu concentrations in the liver, kidney and plasma of rats receiving the Cu-supplemented diet decreased slightly as the Fe intake increased (see Table). However, increased Fe intake did not influence Cu levels in the Cu-deficient rats. Liver Fe increased in the Cu-deficient rats and also in the Fe-supplemented rats receiving the diet with 3 mg Cu/kg but there was surprisingly, no additive effect of low Cu and high Fe supply.

Dietary Cu content (mg/kg diet)	<1	<1	<1	3	3	3
Dietary Fe content (mg/kg diet)	50	500	1000	50	500	1000
Liver Cu (mg/kg)	1.1	1.2	1.0	3.5	3.1	3.0
Kidney Cu (mg/kg)	2.4	2.2	2.4	8.3	4.5	4.5
Plasma Cu (mg/l)	0.06	0.06	0.06	0.90	0.69	0.73
Liver Fe (mg/kg)	380	403	368	92	136	231

In another experiment designed to examine the effect of Fe supply on the apparent absorption of an oral dose of ^{64}Cu , 18 rats were given a diet with 3 mg Cu/kg and 5, 50 or 500 mg Fe/kg for 2 weeks. They were given a single oral dose of ^{64}Cu , killed 3 h later and the ^{64}Cu content of the gut-free carcass, liver and kidneys was determined by whole-body counting. Apparent absorption of ^{64}Cu by the rats receiving 5, 50 and 500 mg Fe/kg diet was 31.5 ± 1.8 , 23.8 ± 1.1 and $26.1 \pm 2.3\%$, indicating that increased Fe intakes did not influence apparent ^{64}Cu absorption, although deficient Fe supply may have had a slight enhancing effect. Variation on Fe supply did not influence the distribution of ^{64}Cu within the carcass.

It appears therefore that rats are much less susceptible than calves to the effects of increased Fe supply and that there are no additive effects of high Fe and low Cu intakes with respect to Cu status or hepatic Fe levels. This may suggest that events occurring within the rumen are involved in the Cu-Fe interaction in calves. Alternatively, it may be that the effect of Fe is principally on the excretion of stored Cu.

Humphries, W. R., Young, B. W., Phillippo, M. & Bremner, I. (1981). *Proc. Nutr. Soc.* **40**, 68A.

Species differences in the retention of orally administered cupric oxide 'needles' in the alimentary tract. By N. F. SUTTLE and E. VALENTE, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

Cupric oxide 'needles' (CuO_N) are retained in the ovine abomasum after oral dosing and slowly release absorbable Cu (Dewey, 1977). In view of the potential of this development in trace element supplementation, investigations were made of the retention of CuO_N by pigs and cows. Two groups of three male pigs, weighing 30–40 kg and receiving a commercial diet unsupplemented with Cu, were given 1.6 g Cu as CuO in 'needle' or powder (CuO_P) form as a single oral dose in gelatin capsules 1 h after feeding. They were housed in metabolism crates and faeces were collected in plastic dustbins. The extent of CuO retention was assessed by monitoring faecal Cu excretion which reached peak levels 2 d after dosing in animals given CuO_P and remained elevated for 6 d (Table 1). In animals given CuO_N , concentrations reached a peak after 4 d and remained elevated for 18 d. Retention of the particulate CuO_N was, however, less pronounced than that found in sheep (Suttle, 1981).

Table 1. *Mean faecal Cu concentration (mg/kg DM) in two groups of three pigs after the oral administration of 1.6 g Cu as cupric oxide in 'needle' (CuO_N) or powder (CuO_P) form*

Period of expt (d)	0	1	2	3	4	5	6	7	8–9	10–11	15–18
CuO_N	155	239	663	1586	2483	1755	433	869	322	242	188
CuO_P	165	390	2676	1597	377	—	196	179	151	159	158

Three adult Jersey cows, weighing 315–470 kg and receiving hay *ad lib.* were given 0.33 g CuO_N /kg body-weight orally in a cellulose Soxhlet extraction thimble. Two animals were slaughtered after 7 d and one after 15 d. Distribution of CuO_N in the alimentary tract was assessed by mixing the contents of a particular organ with water, decanting, sieving the heavy residue with a kitchen sieve (36 holes/cm²) and hand-picking the 'needles' from the coarse residue. In each animal 'needles' were found predominantly in the reticulum (29–53% recovery) and few were present in the abomasum (3–18%). CuO_N were therefore retained by cattle though in a different location from that reported in sheep. Species differences in the anatomy of the alimentary tract may influence the effectiveness of particulate, slow-release sources of trace elements.

Dewey, D. W. (1977). *Search* 8, 326.

Suttle, N. F. (1981). *Vet. Rec.* 108 (In the Press).

Influence of dietary sulphide on the response of rats to ammonium tetrathiomolybdate. By C. F. MILLS, N. T. DAVIES, H. G. WILSON and I. BREMNER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

There are indications that structurally related derivatives of the tetrathiomolybdate ion (MoS_4^{2-}) synthesized within the rumen may be involved in the antagonistic action of dietary molybdenum and sulphur upon the utilization of copper by ruminants (Dick *et al.* 1975; Mills *et al.* 1978). However, it is also evident (Gawthorne & Nader, 1976) that dietary Mo prolongs the biological half life of the sulphide (S^{2-}) pool within the rumen. Even if it were assumed that, in the latter study, all dietary Mo was converted to MoS_4^{2-} , this would have accounted for less than one quarter of the observed effect of Mo on S^{2-} turnover. Accordingly, we have investigated the effects of MoS_4^{2-} on S^{2-} tolerance and metabolism with the particular object of investigating secondary consequences relevant to defective utilization of Cu.

Thirty-six weanling male rats were given a semi-synthetic diet containing 3 mg Cu/kg. Groups of six received supplements providing MoS_4^{2-} (to provide 3 mg Mo/kg diet), calcium sulphide (96 or 288 mg S^{2-} /kg) or both MoS_4^{2-} and CaS for 34 d.

Compared with rats given unsupplemented diet, administration of S^{2-} alone did not influence growth or liver Cu and Fe, heart Cu or blood haemoglobin concentrations. Although the higher dietary concentration of S^{2-} depressed plasma Cu significantly, no clinical signs of Cu deficiency were evident. Treatment with MoS_4^{2-} alone slightly inhibited growth ($0.05 < P < 0.1$) and depressed liver and kidney Cu. Hepatic retention of Fe increased but there were no clinical signs of Cu deficiency.

Simultaneous administration of MoS_4^{2-} with 96 or 288 mg S^{2-} /kg diet dramatically inhibited growth, anaemia developed and animals ultimately lost weight. Traces of sulphaemoglobin were evident in erythrocytes and skeletal defects developed. Effects of MoS_4^{2-} on liver Cu and Fe were exacerbated by dietary S^{2-} ; defective melanogenesis in hair and the development of cardiomegaly provided pathological evidence of an induced Cu deficiency.

These results suggest that traces of MoS_4^{2-} in the diet inhibit the normally rapid oxidation of exogenous sources of S^{2-} and that this effect has secondary consequences upon the utilization of Cu.

Dick, A. T., Dewey, D. W. & Gawthorne, J. M. (1975). *J. agric. Sci., Camb.* **85**, 567.

Gawthorne, J. M. & Nader, C. J. (1976). *Br. J. Nutr.* **35**, 11.

Mills, C. F., Bremner, I., El-Gallad, T. T. T., Dalgarno, A. C. & Young, B. W. (1978). *Trace Element Metabolism in Man & Animals* **3**, 150.

The effect of frequencies of feeding and magnesium supplementation on the efficiency of absorption of magnesium by sheep. By A. C. FIELD, R. A. DINGWALL and C. S. MUNRO, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

Recent studies have shown that magnesium is absorbed from the stomach region of ruminants by a saturable active transport mechanism, possibly influenced by the concentration of Mg, pH or volatile fatty acid concentration and Na:K value in ruminal fluid (see Field, 1980). Some of these factors are subject to prandial variation and it is conceivable that the efficiency of absorption is modified by the frequency of feeding and Mg supplementation; Mg salts are given continuously or discretely for the prevention of hypomagnesaemic tetany in ruminants. This aspect was investigated in two experiments. In the first experiment adult sheep were given 1 kg/d of a complete pelleted diet (2.3 g Mg/kg DM) in a single feed (S) or distributed over 24 h (C) and in the second, similar sheep were given 0.8 kg/d of the same diet in two equal feeds at 09.00 and 16.00 hours with or without 4 g Mg as Mg Cl₂ given in a single dose (S) at 09.00 hours or infused continuously (C) into the rumen. The rate of secretion of Mg in urine was used as the criterion of Mg absorption. On the last day of a trial, urine was collected during successive intervals over 24 h. Absorption of Mg salts was taken as the difference in urinary Mg between the basic diet with and without the Mg supplements. The value for the basic diet was the urinary Mg plus endogenous faecal Mg excretion (Agricultural Research Council, 1980).

In Expt 1 the rate of urinary Mg excretion was constant during the day in both groups, despite large prandial increases in Mg concentration (46 to 172 mg/l) and falls in pH (6.90 to 5.65) and Na:K value (5.74 to 3.03) in ruminal fluid in group S. The pH (6.2), Mg concentration (89 mg/l) and Na:K value (3.70) in ruminal fluid were constant in group C.

In Expt 2 a large prandial variation was seen in ruminal concentration of Mg (138 to 477 mg/l) in group S, but there was no corresponding increase in urinary Mg excretion or plasma Mg. This observation may reflect the fact that ruminal Mg concentrations were generally higher than those associated with saturation of the absorption mechanism in the rumen (150–200 mg/l; Field, 1980).

No effect of frequencies of feeding or Mg supplementation on absorption of Mg was observed; urinary Mg excretion in S and C groups were respectively 0.48 and 0.49 in Expt 1 and 0.95 and 0.93 g/d in Expt 2. Large individual variation in efficiency of absorption of dietary Mg, but not of Mg salts was found; the efficiencies were 0.322 ± 0.0271 (range 0.20–0.40) and 0.155 ± 0.0125 (range 0.14–0.19) respectively.

Agricultural Research Council (1980). *The Nutrient Requirements of Farm Livestock No. 2. Ruminants*. London: Commonwealth Agricultural Bureau.

Field, A. C. (1980). In John Lee Pratt International Symposium, *The Role of Magnesium in Animal Nutrition*. Blacksburg: Virginia Tech.

Gum arabic metabolism in the rat colon. By A. H. McLEAN ROSS, L. F. MCKAY, A. BUSUTTIL, D. M. W. ANDERSON, W. G. BRYDON and M. A. EASTWOOD, *Gastrointestinal Unit and Pathology Department, Western General Hospital, and Department of Chemistry, University of Edinburgh*

Fibre is a complex heterogeneous material increasingly used in the management of colorectal and other diseases (Heaton, 1978). It is now appreciated that cereals, bran, and vegetable fibre behave differently along the gastrointestinal tract (Stephens & Cummings, 1980). Indirect evidence suggests the caecum is a major site for metabolism of certain fibres.

We have studied the metabolism of a chemically defined, readily identifiable polysaccharide, gum arabic (GA) in the rat intestine. GA is a water soluble polysaccharide (molecular weight approximately 850 000) which contains rhamnose, arabinose, glucuronic acid and galactose. It is used as an ingredient in some foodstuffs such as confectionery industry.

Incubation experiments using human pancreatic and gastric juices demonstrate no degradation of the molecule after 48 h.

Wistar rats (3-month-old) were fed on gum arabic, incorporated in pellets of reconstituted oxid breeders diet, at doses of 0–200 g/kg, for a period of 4 weeks. Other rats were fed on a complete elemental diet (residue free) containing gum arabic (220 g/kg dry weight) in jelly form administered over the same time period.

Intestinal contents were examined for precipitable GA by the addition of acidified ethanol. GA was found from stomach to small intestine but not in the caecum, colon, or rectum. Caecal excision and reconstitution of intestinal continuity resulted in GA recovery from stomach to rectum.

Excreted methane, hydrogen, and intestinal volatile fatty acids (VFA) were measured as indicators of bacterial activity in the caecum and colon.

Methane excretion, a measure of caecal bacterial metabolism, increased significantly ($P < 0.001$) on the GA pellet diet. Hydrogen concentrations remained unaltered. Methanogenesis ceased on elemental diets alone or gum supplemented and following caecal excision.

Faecal VFA concentrations increased in a linear fashion with increasing doses of GA ($r = 0.860$). Acetate concentrations increased ($r = 0.972$) and butyrate concentrations decreased ($r = 0.888$) with increasing GA dosage. Significant decreases in concentrations of VFAs were found from caecum to left colon ($P < 0.005$) and from (L) colon to faeces ($P < 0.05$) on control diet but not on GA pellet diet. It can be concluded that (1) GA degradation occurs in the caecum and is associated with increased methane excretion, increased VFA concentration and changes in the proportions of various VFAs in the faeces. (2) Changes in caecal environment alter these relationships with GA metabolism.

Heaton, K. W. (ed.) (1978). *Dietary fibre. Current developments of importance to health.* J. B. Libby & Co. Ltd.

Stephens, A. M. & Cummings, J. H. (1980). *Nature, Lond.* **284**, 5753.

The influence of pentose on breath methane excretion. By LINDA F. MCKAY, W. G. BRYDON, M. A. EASTWOOD and J. H. SMITH, *Wolfson Gastrointestinal Laboratory, University Department of Medicine, Western General Hospital, Edinburgh*

Methane is produced in man by anaerobic bacterial metabolism in the colon and is excreted in flatus and expired breath. Approximately one-third of the adult population excrete methane in the breath (Bond *et al.* 1971).

In this investigation the incidence of methane production in fifty-six healthy subjects aged from 16 to 79 years has been studied in relation to various components of their diet. The mean daily dietary intakes were (\pm SD) 68 ± 19 g protein, 99 ± 32 g fat, 226 ± 83 g carbohydrate, 8.9 ± 2.9 MJ energy and 13 ± 4.9 g fibre. Of the subjects, thirty-four produced methane in concentrations which ranged from 0.09 to 1.49 $\mu\text{mol/l}$ above room concentrations (mean 0.7 $\mu\text{mol/l}$). Breath methane concentrations in methane producers significantly correlated with intakes of the pentose fraction of non-cellulosic polysaccharide (r 0.44, $P < 0.01$) and lignin (r 0.40, $P < 0.05$). The intake of lignin and pentose were correlated (r 0.82, $P < 0.001$). Subsequently, twelve methane producers aged from 28 to 50 years were given test meals containing 5 g pentose as 510 g cooked fruit and vegetables; 385 g orange; 22.1 g bran. Other sources of pentoses 10 g xylan; 25 g D+xylose; 25 g L-xylose; 20 g L+arabinose or 20 g D-arabinose were given to fasting subjects. Breath methane concentrations were measured before the test meal and every half hour for 5 h. Measurements were also taken during a control period of fasting.

The acute administration of complex polysaccharide sources rich in pentose had no significant effect on breath methane production over 5 h. The administration of D+xylose and L+arabinose led to a significant increase ($P < 0.01$; $P < 0.025$ respectively, Wilcoxon Signed Rank Test) in methane excretion both at 90 to 120 min compared to base line and at 210 to 240 min compared to the measurement 1 h previously. Both L-xylose and D-arabinose caused diarrhoea.

The first peak of the biphasic methane response after ingestion of pentose monomers is probably caused by the fermentation of the unabsorbed fraction of pentose passing into the caecum.

The association between breath methane concentration and the ingestion of pentose containing fibre is the result of a steady metabolic state in the caecum. The lack of gas production following acute complex polysaccharide administration could be due to a relatively slow metabolic response of bacteria. The release and availability of free pentose monomers from plant polysaccharides may be rate limiting steps in this process.

The authors wish to acknowledge a Medical Research Council grant (LFM).

Bond, J. H., Engel, R. R. & Levitt, M. D. (1971). *J. exp. Med.* **133**, 572.

The digestion of plant cell wall material in an artificial rumen (Rusitec).

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It was previously reported (Brice & Morrison, 1981) that under the conditions employed, xylanase (hemicellulase) activity determined in Compartment 2 (washings of digesta) of Rusitec (Czerkawski & Breckenridge, 1977) was not a limiting factor in the digestion of plant cell wall material. The substituent groups attached to the hemicellulose chains, e.g. L-arabinose, phenolic acids and acetyl groups together with the possible linkages between hemicelluloses and lignin, are probably more important in limiting the digestion of plant cell wall material (Bacon, 1979).

The experiment reported here was conducted to follow changes in cell wall composition together with changes in the activities of enzymes associated with cell wall digestion, when hay stem material was incubated in Rusitec. The vessel was continuously infused with a solution which contained minerals, casein amino acids but no carbohydrates. The vessel contained two large bags (4 g) and ten small bags (0.2 g) of chopped hay (10 mm) which had been washed with warm water to remove soluble sugars and then freeze-dried. At the start of the experiment the vessel was inoculated with a mixture of filtered rumen liquor and the washings of solid rumen contents.

The small bags were removed from the vessel at fixed time intervals and washed with artificial saliva. These washings were centrifuged at 2000 g for 45 min and the clear supernatant used as the enzyme preparation.

The xylanase activity was found to decrease initially to a minimum after 6 h but then rose to a maximum at 28 h. Cell wall digestion when measured by loss of dry weight was not apparent until 10 h after inoculating the vessel. At 10 h there was a 5% loss of dry weight which rose to 32% by 46 h. However, from 3 to 46 h incubation there was a noticeable and constant increase in the xylose:arabinose value in the undigested residue. At 3 h the value was 6.4 and had risen to 8.2 by 46 h. Whether this was due to the enzymatic removal of the substituent L-arabinose groups attached to the xylan chains has not been determined but α -L-arabinofuranosidase activity was found in the cell wall washings.

Bacon, J. S. D. (1979). *Rep. Rowett Inst.* **35**, 99.

Brice, R. E. & Morrison, I. M. (1981). *Proc. Nutr. Soc.* **40**, 24A.

Czerkawski, J. W. & Breckenridge, G. (1977). *Br. J. Nutr.* **38**, 371.

Biochemical signs of human selenium depletion. By G. S. FELL, P. STROMBERG, A. MAIN, R. SPOONER, R. CAMPBELL and R. RUSSELL, *Royal Infirmary, Glasgow* and A. BROWN and J. M. OTTAWAY, *Strathclyde University, Glasgow*

Biochemical indices of selenium (Se) status were measured in a local reference population and in patients with defined gastrointestinal disease. Values for plasma Se, urine Se/g creatinine, red cell and plasma glutathione peroxidase (GSHPx) are given in the Table.

(Values are means with their standard deviations; no. of subjects in parentheses)

	Plasma Se ($\mu\text{g/l}$)		Urine Se ($\mu\text{g Se/g creatinine}$)		GSHPx activity			
	Mean	SD	Mean	SD	Red cell (U/g Hb)		Plasma (U/l)	
					Mean	SD	Mean	SD
Reference population	107 (52)	19.8	24 (16)	8.5	43 (116)	12.5	459 (107)	85
Coeliac disease	88 (20)	24.8***	18 (20)	11.8	39 (20)	14.4	410 (20)	135**
Ulcerative colitis	113 (19)	26	25.8 (17)	14.2	51 (19)	12	514 (18)	90
Crohn's disease	95 (17)	32*	26 (18)	14	38 (18)	18	417* (18)	128

Values significantly lower than reference by *t* test (Wilcoxon signed-rank test); * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

The coeliac patients had more abnormalities of Se biochemistry than the other groups, due perhaps to the generalized nature of the disease and its protracted duration. Nine coeliac patients had low urinary Se excretion and of those four also had low plasma Se, three had low red cell GSHPx and six had low plasma GSHPx.

In contrast the ulcerative colitis group had few abnormalities possibly reflecting localization of this disease to the colon and rectum. The Crohn's disease patients included four who had severe generalized disease requiring in-patient hospital treatment. These four patients all had low urine Se output, three also with low plasma Se and red cell GSHPx activity.

Some patients with severe gastrointestinal disease may require supplementary feeding with commercial nutrients. Most of these products are low in Se and will not correct Se depletion without addition of Se to the regimen. (Fell, 1980; Spooner *et al.* 1980).

Fell, G. S., Shenkin, A., Main, A. & Russell, R. (1980). *Proc. Nutr. Soc.* **39**, 36A.

Spooner, R. J., Campbell, R. A., Rumley, A. G. & Stromberg, P. (1980). *Proc. Nutr. Soc.* **39**, 37A.

Variable changes in heavy metal absorption after milk feeding. By J.QUARTERMAN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Adult rats given milk as their only food absorb more lead and other heavy metals than rats given solid food (Kello & Kostial, 1973). This effect of milk has also been observed in human subjects (Saarinen & Simes, 1979) but did not occur when rats were offered solid food as well as milk (Quarterman & Morrison, 1981). In most cases the subjects were given milk for 5 d or longer and it is not known how long after milk feeding is begun that heavy metal absorption increases, nor how long after milk-adapted animals are returned to solid food that heavy metal absorption decreases. If these times of adaptation to milk and solid food are short the consumption of milk between meals could affect the absorption of toxic and essential heavy metals.

Rats in groups of six were given milk as their only food to appetite for times ranging from 4 h (0.2 d) to 11 d. At the end of its period of milk feeding each rat was given by stomach tube about 100 kBq (2.7 μ Ci) ^{203}Pb or ^{65}Zn in 0.2 ml saline (9 g sodium chloride/l) containing 5 μ g Pb/ml as acetate or 5 μ g Zn/ml as sulphate. Each rat was killed 1 h after the dose of isotope and activity measured in blood, soft tissues and gut-free carcass.

Effect of length of milk feeding on retention (fraction of dose/ml blood $\times 10^{-4}$) of ^{203}Pb and ^{65}Zn in blood

Length of milk feeding (d)	0	0.2	0.7	1	2	3	4	5	6	7	8	11	
Retention { Expt 1 (^{203}Pb)	1	4	1	2	39	—	—	2	—	44	—	—	$P < 0.01$
Expt 2 (^{203}Pb)	1	—	—	13	15	99	44	6	13	74	—	—	$P < 0.01$
Expt 3 (^{65}Zn)	—	—	—	—	11	18	20	16	70	28	11	12	$P < 0.01$

The first experiment (Expt 1, see Table) showed large increases in ^{203}Pb absorption after 2 d and 7 d but no increase after 5 d. This variable effect of milk feeding was repeated with ^{203}Pb in Expt 2 and ^{65}Zn in Expt 3 though there were differences in the size of the increases and the length of milk feeding required to produce them.

In Expt 3 two further groups of rats were given milk for 3 d then force-fed 1.5 g semi-purified diet homogenized with 1 ml water 1 h or 3 h before the dose of ^{65}Zn . The fractional retention was then reduced to 6×10^{-4} in both groups.

From this and previous work it seems that milk consumed with or between meals is unlikely to increase heavy metal retention.

Kello, D. & Kostial, K. (1973). *Environ. Res.* **6**, 355.

Quarterman, J. & Morrison, E. (1981). *Proc. Nutr. Soc.* **40**, 25A.

Saarinen, V. M. & Simes, M. A. (1979). *Pediat. Res.* **13**, 143.

Inhibition of arachidonic acid metabolism in the uteri of zinc deficient parturient rats. By S. C. CUNNANE, *Rowett Research Institute, Aberdeen, Scotland AB2 9SB*

Myometrial strips from parturient zinc deficient rats exhibit significantly less spontaneous activity *in vitro* than do those from zinc adequate rats (Cunnane, 1981). This effect may be related to the abnormal parturition and high foetal mortality in Zn deficient rats. Prostaglandins (PGs) are required for normal uterine contractility and parturition and Zn has been suggested to be involved in PG synthesis (Cunnane *et al.* 1980). Therefore, arachidonic acid metabolism to '2 series' PGs was studied in the uteri of parturient Zn deficient rats.

Second parity Hooded Lister rats (Rowett strain) were fed semisynthetic diets containing zinc at 20 ppm (adequate), 5 ppm (chronic deficient) or 10 ppm (2 weeks) then 0.5 ppm (1 week) (acute deficient). On day 22 of gestation the rats were sacrificed and uteri excised, minced in Krebs-Henseleit buffer (pH 7.4) and 25 mg of tissue incubated with [3 H]arachidonic acid for 30 min at 37°. The reaction mixture was then acidified and the lipids extracted with ether-ethylacetate (4:1) and dried under nitrogen. PGs were separated by thin layer chromatography (Merck 5735 plates) and the radioactivity measured in the bands corresponding to arachidonic acid, PGE₂, PGF_{2 α} and 6-oxo-PGF_{1 α} (PGI₂).

Conversion of arachidonic acid to all three PGs (mg tissue/unit time) was inhibited by 50% ($P < 0.01$) in the most Zn deficient group (10 ppm to 0.5 ppm Zn), but was not significantly different from control (20 ppm Zn) in the 5 ppm Zn group. As a percentage of the total '2 series' PGs, significantly less PGF_{2 α} was synthesized in rats on the 10 to 0.5 ppm Zn diet compared to controls but proportional PGI₂ synthesis was increased with decreasing Zn in the diet ($P < 0.01$).

It is concluded that the decreased conversion of arachidonic acid to '2 series' PGs correlates directly with the low *in vitro* spontaneous activity of myometria from Zn deficient rats and that both these factors contribute significantly to the high perinatal mortality observed at parturition in Zn deficient rats. Furthermore, Zn appears to be a regulatory factor in the metabolism of arachidonic acid to '2 series' PGs.

Cunnane, S. C. (1981). *Proc. Nutr. Soc.* **40**, 80A.

Cunnane, S. C., Huang, Y. S., Horrobin, D. F. & Davignon, J. (1980). *Prog. Lipid Res.* (In the Press).

The effect of fluoride on plastic deformation and collagen content of rat bones. By JOAN E. ALLIBONE (introduced by J. QUARTERMAN), *Rowett Research Institute, Aberdeen, and the Department of Agriculture, University of Aberdeen*

The effect of fluoride on bones varies according to the dietary treatment and the physiological state of the subject. In rats there are few effects on the mineral composition of bones but a number of changes in the organic matrix including collagen have been reported (Allibone, 1980). Evidence with respect to the effects of F on collagen synthesis and on the production of abnormal collagen is conflicting but there is little information on the total collagen produced in fluorotic rats in relation to the physical properties of the bones.

In a previous experiment (Quarterman *et al.* 1979) the addition of fluoride (100 µg/g) to a semi-purified diet given to rats from weaning to 3 months of age decreased the breaking stress, elasticity and hydroxyproline content (µg/g fresh weight) of their femurs and tibias. A further study has now been made with the same semi-synthetic diets but with 200 µg F/g for 5 weeks from 5 weeks of age. The breaking stress and elasticity of femurs and tibias were found to be decreased as before. Bones of rats given fluoride exhibited more extensive plastic deformation and there was a greater degree of bending/unit applied force before fracture than in rats without fluoride.

Collagen was extracted from decalcified femurs with guanidine hydrochloride and estimated as hydroxyproline (Rucker *et al.* 1975). Dietary fluoride increased the total collagen content of the bones by about 11% but had no consistent effect on soluble (non-centrifugable) collagen.

It is not known if the decreased bone strength is related to the increased collagen content but this work does suggest that the early stages, at least, of collagen polymerization are not greatly affected by fluoride.

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Quarterman, J., Morrison, J. N., Morrison, E. & Mills, C. F. (1979). *Proc. Nutr. Soc.* **38**, 84A.

Rucker, R. B., Riggins, R. S., Laughlin, R., Chan, M. M., Chen, M. & Tom, K. (1975). *J. Nutr.* **105**, 1062.

Decreased spontaneous uterine contractility at parturition in zinc deficient rats. By S. C. CUNNANE, *Rowett Research Institute, Aberdeen, Scotland AB2 9SB*

Conversion of arachidonic acid to '2 series' prostaglandins has been shown to be decreased by 50% in the uteri of zinc deficient parturient rats (Cunnane, 1981). Severe Zn deficiency also causes high perinatal mortality and may prolong as well as delay parturition (Apgar, 1977). These effects suggest defective uterine function at parturition in Zn deficient rats, a possibility which was studied in vitro using uterine strips.

Second parity Hooded Lister rats (Rowett Strain) were maintained throughout gestation (22 d) on a semi-synthetic diet containing various amounts of Zn: 20 ppm (adequate), 10 ppm (marginal), 5 ppm (chronic low), or 10 ppm (15 d) then 0.5 ppm (7 d) (acute low). On day 22 of gestation (within 12 h of parturition) the rats were sacrificed and the uteri excised. Longitudinal strips (approximately 2 × 12 mm) from both horns were placed in muscle baths and connected to force displacement transducers. The surrounding medium was oxygenated Krebs phosphate buffer (pH 7.4) maintained at 37°. The buffer was washed out at 20 min intervals and the uterine strips allowed to contract spontaneously for 1 h.

No significant long-term changes in baseline tension occurred during the experimental period in any of the preparations. Contractility was divided into (1) an amplitude component (<5 mm, 5–10 mm or >10 mm where 5 mm = 3 g tension), (2) a frequency component. The frequency of low amplitude contractions (<5 mm) was inversely proportional to dietary Zn concentration ($P < 0.01$). Frequency of medium amplitude concentrations (5–10 mm) did not significantly correlate with dietary Zn, whereas the frequency of high amplitude contractions (>10 mm) correlated directly with dietary Zn concentration ($P < 0.01$). High amplitude spontaneous contractions were characteristically absent in uteri from low Zn rats, an effect which could not be restored by addition of oxytocin (5 mU/ml) or prostaglandin $F_{2\alpha}$ (1–10 ng/ml) to the buffer.

It is concluded that spontaneous uterine contractility is very significantly depressed in parturient Zn deficient rats, an observation which may be due to the decrease in synthesis of prostaglandins E_2 , $F_{2\alpha}$ and I_2 in the uteri of these rats.

Apgar, J. (1977). *J. Nutr.* **107**, 1399.

Cunnane, S. C. (1981). *Proc. Nutr. Soc.* **40**, 78A.

Dietary sunflowerseed oil, insulin secretion and $\Delta 9$ -desaturase activity in obese and lean Zucker rats. By T. E. C. WEEKES,¹ K. W. J. WAHLE² and M. B. LEBAIJURI,¹ ¹*Department of Agricultural Biochemistry and Nutrition, University of Newcastle, Newcastle upon Tyne NE1 7RU* and ²*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Fatty acid $\Delta 9$ -desaturase activity in rat liver and adipose tissue is depressed in diabetes and when high levels of polyunsaturated fatty acids are fed (Worcester *et al.* 1979). The possibility that changes in insulin secretion may mediate the effects of sunflowerseed oil on fatty acid desaturation was investigated using male Zucker obese rats (*fa/fa*) and lean littermates (*Fa/-*).

Animals were fed *ad lib.* for a minimum period of 27 d on purified diets containing either 50 (LS) or 200 (HS) g sunflowerseed oil/kg diet.

In Expt 1, two groups were used, each of eight obese rats. The HS diet resulted in a markedly lower specific activity of $\Delta 9$ -desaturase in liver microsomes ($P < 0.001$), and in a higher plasma insulin concentration ($P < 0.05$) than on the LS diet.

In Expt 2, the value for the ratio fatty acids 16:1/16:0 + 16:1 in liver lipid (DESI) was used as an indirect index of desaturation. Insulin release from pancreas fragments was measured *in vitro* on stimulation with glucose (3 g/l). The mean values (\pm SEM) are shown in the Table. Differences between genotypes were significant in all cases ($P < 0.001$). DESI was not related to either plasma insulin or insulin release *in vitro*. However, strong inverse relationships were found between DESI and the proportion of the fatty acid 18:2 ω 6 in liver lipid (lean $r = -0.848$; $P < 0.001$, obese $r = -0.776$; $P < 0.01$). A similar relationship has been described in mice (Enser, 1979).

	Lean				Obese				Effect of diet
	LS		HS		LS		HS		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Plasma insulin† (mU/l)	48	(11)	35	(5)	279	(40)	433	(81)	NS
Insulin release <i>in vitro</i> (mU/min per kg)	364	(93)	302	(72)	632	(106)	685	(110)	NS
Liver lipid composition (g/kg lipid):									
16:1	43	(4)	15	(3)	132	(13)	44	(4)	***
18:2 ω 6	128	(12)	292	(13)	47	(4)	232	(11)	***
DESI	0.158	(0.014)	0.079	(0.011)	0.315	(0.035)	0.149	(0.014)	***

NS, not significant.

*** $P < 0.001$.

†Six observations per treatment.

Enser, M. (1979). *Biochem. J.* **180**, 551.

Worcester, N. A., Bruckdorfer, K. R., Hallinan, T., Wilkins, A. J., Mann, J. A. & Yudkin, J. (1979). *Br. J. Nutr.* **41**, 239.

The effect upon the nutritional value of soya-bean meal of incubation in the bovine rumen. By J. A. ROOKE, S. J. SEYMOUR and D. G. ARMSTRONG, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, NE1 7RU*

The portion of feed protein that escapes degradation in the rumen has been assumed to be equivalent to the original feed protein in nutritional value (Roy *et al.* 1977).

To test this assumption, untreated and formaldehyde-treated soya-bean meals were each incubated for 2 or 24 h in polyester bags in the rumens of four Jersey cattle receiving a diet at 1.4 × maintenance of (g/kg DM intake) hay 267, rolled barley 592, untreated soya-bean meal 119. The diet supplied 25 g N/kg DM. The bags were washed on removal from the rumen and freeze-dried. Each of the soya-bean meals and each of the residues remaining after incubation in the rumen were incorporated as sole N sources into diets for rats providing 15.7 g N/kg DM. The diets were fed for a 14 d period comprising a 7 d N balance trial (five rats/diet) following a 7 d preliminary period. A diet containing 80 g egg albumen/kg DM intake was included as control and endogenous urinary N and metabolic faecal N were determined from a diet containing 20 g egg albumen/kg DM intake.

Biological value and true digestibility of soya-bean meal N after incubation in situ in the bovine rumen

Soya-bean meal:	Time in rumen (h)	Biological value		True digestibility	
		Mean	SE	Mean	SE
Untreated	0	63.6 ^{ab}	1.84	86.3 ^b	0.50
	2	62.7 ^{ab}	0.46	89.8 ^{cd}	0.45
	24	65.7 ^b	2.57	89.3 ^{cd}	0.27
Formaldehyde-treated	0	59.7 ^{ab}	3.97	83.3 ^a	1.52
	2	66.8 ^b	1.88	88.3 ^{bc}	1.80
	24	57.5 ^a	2.38	92.5 ^d	0.57
Egg albumen	—	97.8 ^c	0.41	97.8 ^e	0.29

a,b,c,d,e, Means in the same column with different superscripts are significantly different ($P < 0.05$).

The Table shows that incubation in the rumen for periods of 2 or 24 h did not change the BV of the soya-bean meals but the true digestibility of the residues was significantly increased when compared with the original soya-bean meal, whether formaldehyde-treated or not. Clearly, the nutritive value of the soya-bean residues was not adversely affected by exposure to ruminal degradation.

Roy, J. B., Balch, C. C., Miller, E. L., Ørskov, E. R. & Smith, R. H. (1977). In *Protein Metabolism & Nutrition*, E.A.A.P. Publication no. 22 p. 126 [S. Tamminga, editor]. The Netherlands: Wageningen.

Digestion of formaldehyde treated soya-bean meal in the bovine rumen.

By J. A. ROOKE, B. W. NORTON* and D. G. ARMSTRONG, *Department of Agricultural Biochemistry and Nutrition, University of Newcastle upon Tyne, NE1 7RU*

Four Jersey cattle, each equipped with a rumen fistula and re-entrant cannula in the proximal duodenum were fed ($1.4 \times$ Maintenance) on a basal diet (B) comprising 3 parts hay to 6.8 parts rolled barley (DM basis) and supplying 17.2 g nitrogen/kg DM. Subsequently, using a Latin Square design each of the animals received each of four diets in which 65 or 130 g/kg of B were replaced with untreated soya-bean meal or formaldehyde-treated soya-bean meal (3.0 g HCHO/kg meal treated) to give N intakes of 21.1 and 25.0 g N/kg DM.

Fate of ingested N (g/kg $W^{0.75}$ per 24 h)

	Diets					SEM
	Basal	Low-N		High-N		
		Untreated	Treated	Untreated	Treated	
Apparent N digestibility	0.581 ^a	0.634 ^{abc}	0.615 ^{ab}	0.691 ^c	0.684 ^{bc}	0.0140
Total non-ammonia nitrogen (NAN) entering small intestine	1.070 ^a	1.296 ^b	1.369 ^{bc}	1.389 ^{cd}	1.422 ^d	0.0494
Microbial NAN entering small intestine	0.803 ^a	1.063 ^b	1.062 ^b	1.089 ^b	0.981 ^{ab}	0.0408
Feed NAN entering small intestine	0.267 ^a	0.233 ^a	0.307 ^a	0.300 ^a	0.441 ^b	0.0262
NAN entering small intestine (g/g N intake)	1.303 ^b	1.060 ^a	1.107 ^b	0.946 ^a	0.971 ^a	0.0423

a,b,c,d, Values with different superscripts on same line differ significantly ($P < 0.05$).

It can be seen from the Table that replacement of untreated soya-bean meal with HCHO-treated product on an equal N basis gave no significant differences in apparent digestibility of N. The inclusion of protein concentrates increased the daily entry of non-ammonia N (NAN) into the small intestine and the flows were higher for the higher level of protein concentrate included. There was no significant difference in flows between the two forms of meal when fed at the same level. These increased flows were associated with increasing amounts of feed NAN passing the rumen undegraded, although at the higher level of supplementation with the HCHO-treated protein the effect was balanced by a decrease in flow of microbial NAN. Mean net efficiency of microbial N synthesis in the rumen was 34.6 ± 1.40 g N/kg OM apparently digested in the rumen. Feed N degradabilities for the untreated and HCHO-treated proteins were 0.74 ± 0.180 and 0.39 ± 0.061 respectively.

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Effects of fasting on substrate utilization during exercise in man. By R. J. MAUGHAN and C. WILLIAMS, *Department of Surgery, University of Aberdeen and Department of Physical Education and Sports Science, Loughborough University*

It has been proposed that substrate utilization in muscle is regulated by a glucose-fatty acid cycle (Randle *et al.* 1963). The cycle depends on the fact that alterations in the rate of fat oxidation by muscle cause changes in the cytoplasmic citrate concentration. Citrate has an inhibitory effect on phosphofructokinase (PFK) which can, under certain circumstances, regulate the rate of flux through the glycolytic pathway. The present study is an attempt to establish whether this mechanism is operational in human skeletal muscle.

Substrate and metabolite levels in blood and skeletal muscle were measured before and after exercise in four healthy young adult male subjects in a fed state and again after a 24 h fast. Exercise consisted of 15 min of work on a bicycle ergometer at a work load corresponding to 50% of maximum oxygen uptake. The respiratory exchange ratio (R) was lower (0.86 ± 0.02 ; mean \pm SEM) in the fasted state than in the fed state (0.94 ± 0.03 ; $P < 0.005$), indicating that the rate of fat oxidation was higher in the fasted subjects and the rate of carbohydrate oxidation correspondingly lower.

Blood metabolite concentrations (mmol/l)

(Values are means with their standard deviations; no. of subjects in parentheses)

	Fed				Fasted			
	Pre-exercise		Post-exercise		Pre-exercise		Post-exercise	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glucose	(4) 4.96	0.47	5.46	0.59	4.61	0.37	5.19	0.43
Lactate	(4) 1.42	0.67	1.30	0.57	0.85	0.15	1.65	0.84
Pyruvate	(4) 0.061	0.027	0.059	0.029	0.057	0.016	0.072	0.026
Plasma free fatty acids	(4) 0.267	0.120	0.279	0.144	0.499	0.129	0.852	0.368
β -OH butyrate	(4) 0.101	0.022	0.115	0.023	0.313	0.091	0.270	0.074
Acetoacetate	(2) 0.013		0.017		0.134		0.095	

There was a non-significant decrease in muscle glycogen content during exercise in both fed and fasted states. The decreased carbohydrate oxidation in the fasted state appeared to result from a reduced contribution of blood glucose to metabolism rather than a decrease in the amount of muscle glycogen utilized. Muscle citrate content increased non-significantly during exercise in both the fed and fasted state. There was, however, no evidence of a significant change in the muscle content of hexose monophosphates during exercise or in response to fasting. This suggests that citrate-mediated inhibition of glycolysis at the level of PFK was not taking place under the conditions of this experiment.

Randle, P. J., Garland, P. B., Hales, C. N. & Newsholme, E. A. (1963). *Lancet* i, 785.

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Differential effects of fasting on skeletal muscle glycogen content in man and on skeletal and cardiac muscle glycogen content in the rat. By R. J. MAUGHAN and C. WILLIAMS, *Department of Surgery, University of Aberdeen, Aberdeen and Department of Sport and Recreation Studies, Loughborough University, Loughborough*

Macleod & Prendergast (1921) reported that fasting had little effect on the skeletal muscle glycogen content, but caused an increase in the myocardial glycogen content in the rabbit and the dog. Since then, conflicting reports on the effects of fasting on muscle glycogen content have been produced. In an attempt to resolve these differences, the effects of fasting on the muscle glycogen content have been re-evaluated. Samples of skeletal muscle were obtained by needle biopsy from the m. vastus lateralis of seven healthy young adult males following a light breakfast and again following a 24 h fast.

Male rats (body-weight 250–300 g) were allocated to either a fed group (n 8) which had unrestricted access to food up to the time of the experiment, or a fasted group (n 6) which was deprived of food for 24 h before the experiment. Animals were anaesthetized and the lateral portion of the left gastrocnemius and the right soleus were exposed and then freeze-clamped *in situ*. The still-contracting ventricles were similarly freeze-clamped *in situ*. Samples were then freeze-dried and the glycogen content determined by enzymatic hydrolysis with subsequent estimation of the liberated glucose.

Muscle glycogen content (mmol glucosyl units/kg dry weight)

	Fed		Fasted	
	Mean	SD	Mean	SD
Human m. vastus lateralis	243.8	59.6	240.3	46.8
Rat soleus	112.3	18.7*	51.4	7.7
Rat gastrocnemius	155.6	15.3*	110.3	6.6
Rat myocardium	111.7	25.0	117.8	18.9

* $P < 0.001$.

The results indicate that there are large differences between the glycogen contents of the different skeletal muscles studied; in the rat, this may reflect differences in muscle fibre composition, the soleus being composed largely of slow-twitch, high oxidative fibres, whereas the lateral portion of the gastrocnemius is comprised mainly of fast-twitch, low oxidative fibres. The m. vastus lateralis of man is of a variable mixed fibre composition.

The failure of the muscle glycogen content to decrease in the human subjects probably reflects the lack of physical activity undertaken by the subjects. The rat cardiac muscle glycogen content did not change, supporting the operation of a glycogen-sparing glucose-fatty acid cycle, in this tissue (Randle *et al.* 1963).

Macleod, J. J. R. & Prendergast, D. J. (1921). *Trans. Roy. Soc. Can., Sect. 5*, 37.

Randle, P. J., Garland, P. B., Hales, C. N. & Newsholme, E. A. (1963). *Lancet* *i*, 785.

Growth in body, skeleton and brain of rats undernourished from birth till 100 d of age. By M. A. WARREN and K. S. BEDI (introduced by J. L. SMART), *Department of Anatomy, Marischal College, University of Aberdeen*

There is a considerable volume of literature on the growth patterns of rats undernourished during the brain growth spurt period; that is, between birth and weaning (Dobbing, 1974). In absolute terms of course, most body growth occurs after weaning. Despite this, few studies have examined rats undernourished beyond this stage. This report concerns such a study. We have been particularly interested in the somatic, skeletal and brain growth of rats undernourished for relatively lengthy periods.

Rat pups were undernourished from day 18 of gestation and during the suckling period by restricting maternal food intake to about half that of control mothers (fed *ad lib.*). They were then placed in randomized pairs, each pair being assigned to a separate cage. Undernutrition of these rats was continued till 100 d of age by restricting their food intake to half that of well-fed age-matched controls. Some rats were subsequently rehabilitated till day 200. All rats received the same good-quality diet (Oxoid Breeding Diet) only the quantity differed in the undernourished groups. Six control and six undernourished rats were killed by perfusion with glutaraldehyde on each of days 0, 3, 6, 12, 18, 25, 50, 100 and 200. The forebrain, cerebellum and brain stem of each rat were dissected out and weighed separately.

The deficit in body-weight in the undernourished rats increased so that by weaning (day 25) it amounted to 53% and by day 100 to 58% compared with age-matched controls. At this age, all the brain regions studied showed substantial deficits in weight. After rehabilitation, the body-weight deficit had fallen to about 25% ($P < 0.01$) and the forebrain and cerebellum also showed significant, persisting deficits. In contrast, the brain stem appeared to show almost complete 'catch-up'.

Radiographic methods were used to analyse the growth patterns of various skeletal features, namely lengths of body, tail, right humerus, right radius, left ulna, left femur, left tibia, right third metatarsal, right hind third proximal phalanx and xygomatic arch and iliac crest widths. Rats examined at various stages during the period of undernutrition had statistically significant deficits in all these dimensions. After rehabilitation, the previously undernourished rats showed 'catch-up' in some, but not all of these features.

Dobbing, J. (1974). In *Scientific Foundation of Pediatrics* pp. 565-577. [J. A. Davies and J. Dobbing, editors]. London: Heinemann.