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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Twenty-second Meeting of the Nutrition Society was held in the School of Agriculture, the University of Nottingham, Sutton Bonington, Loughborough on Friday, 29 *September,* 1978, *when the following papers were read:*

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Gluconeogenesis in isolated sheep hepatocytes. By R. I. RICHARDSON* and ANNE F. LIVESEY†, *Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, Wales SY3 3DD*

Several groups have reported the isolation of ruminant hepatocytes. We report results from hepatocytes isolated from slaughterhouse material, using simple perfusate.

The caudate lobe of an adult sheep was excised as soon as it had been exposed during routine slaughter, the largest visible vein was catheterised and a syringe was used to perfuse 100 ml of ice-cold Ca^{2+}-free and glucose-free Hanks' solution, pH 7·4. The lobe was then perfused, during transport to the laboratory, with approximately 1·5 l of the ice-cold medium, a flow rate of 40–50 ml/min was obtained using gas pressure (95% O_2:5% CO_2). In the laboratory the lobe was connected to the perfusion apparatus and was brought to 37° in 10 min and then 150 ml of fresh medium containing 1·5 g bovine serum albumin and 75 mg Collagenase (type II, Sigma Chemicals, London) was recirculated through the lobe until it began to swell. The lobe was broken open with scissors and shaken in the perfusion medium for 10 min. The cells were then filtered through nylon bolting cloth (61 μm pore size), centrifuged at 100 *g* for 2 min, resuspended in Krebs-Ringer bicarbonate, pH 7·4, and recentrifuged.

Viable hepatocyte preparations were incubated in Krebs-Ringer bicarbonate medium pH 7·4, at 37° under an atmosphere of 95% O_2:5% CO_2. Rates of gluconeogenesis were variable between preparations but could be maintained for over 2 h.

Table 1. *Glucose production in isolated sheep hepatocytes (nmol/mg dry weight of cells per h)*

(All values are the mean of triplicate incubations carried out on hepatocytes isolated from each of four livers with standard errors. Each substrate was at a concentration of 10 mM in the incubation)

Series I			Series II		
	Mean	SEM		Mean	SEM
No substrate	22·4	2·8	Propionate	95·6	44·9
Propionate	48·1	4·1	Propionate + 10^{-8}M-glucagon	108·2	48·8
Pyruvate	81·0	7·7	Propionate + 10^{-6}M-glucagon	121·3	55·6
Alanine	47·2	6·4	Propionate + 10^{-5}M-glucagon	132·0	55·5
Glutamate	30·5	3·2			
Glutamine	26·4	4·0			

There was a greater between-liver variability for cells used in Series II, however, hepatocytes from all four livers showed stimulation of glucose release by glucagon. Only the value for 10^{-5}M-glucagon is significantly greater ($P<0·05$) than that for propionate alone and is similar to that found by Clarke *et al.* (1976) for non-ruminant lambs.

Clarke, M. G., Filsell, O. H. & Jarrett, I. J. (1976). *Biochem. J.* **156**, 671.

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The effect of ammonia on gluconeogenesis by isolated sheep liver cells.

By T. E. C. WEEKES, R. I. RICHARDSON and N. GEDDES, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

Plasma glucose concentrations are reduced when sheep are fed a high-urea diet, suggesting an impairment of gluconeogenesis (Leonard *et al.* 1977). To study the direct effects of ammonia on gluconeogenesis from propionate we used adult sheep hepatocytes, prepared by the method of Richardson & Livesey (1978).

Cells were incubated at 39° in Krebs-Ringer bicarbonate buffer, pH 7·4, for 150 min. The rate of glucose production from 10 mM-propionate remained linear over this period, but varied significantly between experiments ($P<0.001$). Glucose production was significantly reduced by all concentrations of ammonium chloride tested (see table).

Ammonium chloride concentration (mM)	Glucose production (μmol/g protein per h)
0·00	80.0 ± 10.9^{A} (4)
0·25	62.6 ± 10.6^{B} (4)
0·50	45.5 ± 9.3^{C} (4)
0·75	45.3 ± 10.1^{C} (4)
1·00	40.2 ± 10.2^{C} (4)
1·50	37.3 ± 8.8^{C} (3)
2·00	34.1 ± 9.1^{C} (3)

10 mM-propionate present in all flasks. Values are means with standard errors.
Figures in parentheses indicate no. of experiments.
Means with different superscripts are significantly different: $P<0.01$.

Ammonium chloride concentrations of 1 mM and above reduced glucose production to rates not significantly greater than those either in the absence of propionate (30.3 ± 9.3 (4) μmol/g protein/h) or in the presence of 2 mM-ammonium chloride without propionate (31.9 ± 9.0 (4)).

The rate of urea formation estimated by the method of Wybenga *et al.* (1971), was 12.8 ± 0.8 (3) μmol/g protein per h in the absence of both propionate and ammonium chloride. Propionate (10 mM) alone slightly increased this rate (15.1 ± 1.2 (3) μmol/g protein per h), as did 2 mM-ammonium chloride alone (18.5 ± 0.8 μmol/g protein per h) ($P<0.001$). Urea formation was markedly stimulated in the presence of 10 mM-propionate and ammonium chloride together, with rates of 27.1 ± 0.9, 31.0 ± 0.9 and 35.7 ± 0.5 (3) μmol/g protein per h at 0·25, 0·50 and 1·00 mM-ammonium chloride respectively (all $P<0.001$). However, competition for energy (ATP) between glucose and urea formation could only account for a third of the inhibitory action of ammonia on gluconeogenesis from propionate.

Leonard, M. C., Buttery, P. J. & Lewis, D. (1977). *Br. J. Nutr.* **38**, 455.
Richardson, R. I. & Livesey, A. F. (1978). *Proc. Nutr. Soc.* **38**, 2A.
Wybenga, D. R., Di Giorgio, J. & Pileggi, V. J. (1971). *Clin. Chem.* **17**, 891.

Rumen microbial synthesis of long-chain fatty acids. By R. KNIGHT, J. D. SUTTON, J. E. STORRY and P. E. BRUMBY, *National Institute for Research in Dairying, Shinfield, Reading, Berks. RG2 9AT*

Increases in the amount of long-chain fatty acids during passage of digesta through the stomach of sheep suggest that microbial synthesis of fatty acids from non-lipid precursors occurs in the rumen (Knight *et al.* 1977), but direct evidence for such synthesis is confined to in vitro studies (Czerkawski *et al.* 1975).

To identify the origins of the increase in fatty acids more closely, [1–^{14}C]acetate, chosen as a likely precursor on the basis of *in vitro* studies, was infused intra-ruminally for 156 h into three sheep, fitted with rumen and re-entrant duodenal cannulas, given 200 g hay and 400 g concentrates/d. During the last 48 h of infusion, rumen and duodenal microbial preparations were obtained by high speed centrifugation. Duodenal digesta was totally collected for 12 h and a sample retained for analysis. Chromic oxide was used as the marker. Fatty acids were extracted from feeds and digesta by a direct saponification method and quantified by gas-liquid chromatography (Bines *et al.* 1978). The incorporation of [1–^{14}C]acetate into fatty acids was determined by liquid scintillation counting.

The flow of fatty acids to the duodenum (11·8 g/d) exceeded dietary intake (9·4 g/d), the increase in fatty acids (2·4 g/d) consisting mostly of C18:0 and C16:0 in an approximate ratio of 2:1. Microbial lipids contained C18:0 and C16:0 in similar proportions to those observed in the increase in fatty acids in the stomach suggesting that the increase was of microbial origin. On the basis of the incorporation of labelled C into duodenal and microbial lipids it was estimated that 75% of fatty acids passing to the duodenum were of microbial origin, a substantial portion of this undoubtedly representing direct incorporation of dietary lipids rather than *de novo* synthesis. In addition it was estimated that roughly 35% of the increase in fatty acids was accounted for by synthesis in the rumen from acetate. The activity was incorporated largely into C16:0 but surprisingly none was detected in C18:0 and the results offer no explanation for the increase of this acid in the stomach.

The increase in fatty acids in the stomach, 25% of intake, was considerably smaller than previous estimates by ourselves and others, most of which were based on chloroform methanol extraction of lipids, followed by saponification and further extraction of the fatty acids. This technique has been shown to extract fatty acids incompletely from some biological materials, particularly feedstuffs compared with duodenal digesta. Consequently some previous estimates of the magnitude of fatty acid synthesis in the stomach may have been too large.

R. K. acknowledges receipt of an ARC scholarship.

Bines, J. A., Brumby, P. E., Storry, J. E., Fulford, R. J. & Braithwaite, G. D. (1978). *J. agric. Sci., Camb.* **91**, 135.
Czerkawski, J. W., Christie, W. W., Breckenridge, G. & Hunter, H. L. (1975). *Br. J. Nutr.* **34**, 25.
Knight, R., Sutton, J. D. & Storry, J. E. (1977). *Proc. Nutr. Soc.* **36**, 71A.

Combined effects of cafeteria and tube-feeding on energy balance in the rat. By NANCY J. ROTHWELL and M. J. STOCK, *Department of Physiology, Queen Elizabeth College, London W8 7AH*

We have previously shown that tube-feeding rats a fraction of daily energy intake causes obesity by increasing metabolic efficiency, whilst feeding a varied and palatable (cafeteria) diet results in obesity due to hyperphagia. When treatments are withdrawn and animals allowed free access to stock diet, tube-fed rats lose weight as a result of hypophagia whilst cafeteria animals exhibit both hypophagia and an increased resting metabolic rate (Rothwell & Stock, 1978*a*, 1978*b*). Because the mechanisms by which weight is gained and lost differ so markedly, we have now studied the combined effects of cafeteria and tube-feeding.

Adult, male rats were maintained on stock diet (Oxoid) *ad lib.* supplemented with either a variety of food items [C], daily intra-gastric loads (Complan) amounting to 50% of normal energy intake [T], or a combination of both [TC]. After 20 d, groups [C] and [T] were allowed stock diet [S] only whilst in group [TC] withdrawal of either tube-feeding [TC→C], cafeteria diet [TC→T], or both [TC→S] was followed by a further 7 d before all animals were returned to stock diet alone.

Diet	Energy intake day 1–20	g Gained/ MJ eaten day 1–20	Diet	Energy intake day 20–27	Resting $\dot{V}O_2$ day 22	Diet	Energy intake day 27–31	Resting $\dot{V}O_2$ day 29
[T]	100	150*	[S]	89*	100	—	—	—
[C]	125*	117	[S]	81*	110*	—	—	—
[TC]	108*	144*	[TC→S]	55*	112*	[S]	106	100
			[TC→T]	87*	—	[S]	80*	116*
			[TC→C]	107*	—	[S]	70*	120*

All values given as percentage of control values.
*Significantly different ($P<0.05$) from controls.

The results for groups [C] and [T] confirm our previous findings. The combination of tube-feeding with the cafeteria diet [TC] results in excessive weight gains due to an interaction of their individual effects i.e. moderate hyperphagia together with an increased weight gain/MJ eaten. Similarly, the weight loss that occurs when rats are returned to stock diet alone is associated with both hypophagia and an elevated metabolic rate. This increase in thermogenesis is not normally seen in rats recovering from obesity induced by tube-feeding alone (e.g. group [T]) and suggests that it is a specific response to cafeteria-induced obesity, even when the cafeteria diet is withdrawn ([TC→T]) 7 d before recovery commenced.

These results demonstrate that energy balance involves controls operating on both energy intake and expenditure and that their relative contributions are complex and highly susceptible to dietary manipulation.

Rothwell, N. J. & Stock, M. J. (1978*a*). *J. Physiol.* **276**, 60.
Rothwell, N. J. & Stock, M. J. (1978*b*). *Nature, Lond.* **273**, 146.

Effects of tube-feeding iso-energetic amounts of various dietary lipids on energy balance in the rat. By M. I. GURR, *Unilever Research Laboratories, Sharnbrook, Beds.*, NANCY J. ROTHWELL and M. J. STOCK, *Department of Physiology, Queen Elizabeth College, London W8 7AH*

The purpose of this study was to investigate the effects of imposing an additional energy load (by stomach tube-feeding) on voluntary intake, body weight gain and energy expenditure in the rat.

Adult, male rats were fed iso-energetic amounts of either a balanced diet (Complan, Glaxo Ltd.), or various lipids, including corn oil, butter, coconut oil or medium chain triglyceride (MCT), by gastric intubation. The energy delivered by stomach tube represented approximately 40% of normal energy intake, and experimental animals and their free-feeding controls were allowed unlimited access to a semi-synthetic stock diet (Unilever, Bedford).

Rats tube-fed the balanced diet reduced voluntary intake such that total energy intake was the same as control intake but, due to a large (60%) increase in feed conversion efficiency (weight gain/kJ eaten), gained excessive amounts of weight. Those animals intubated with corn oil or butter consumed 14% more energy than controls ($P<0.01$), exhibited a small increase in body weight gain (which was not significant) and an unaltered feed efficiency. In contrast, feeding coconut oil resulted in a greater total energy intake, but a significant reduction in weight gain and metabolic efficiency compared to controls. Resting oxygen consumption of these animals, measured after one or two weeks of treatment, was elevated by 12%. In the group of rats fed MCT, energy intake and body weight gain were slightly depressed, but feed efficiency was reduced by 50%, and these animals displayed a marked increase (25%) in resting oxygen consumption which persisted for 20–24 h after intragastric feeding. Fatty acid analysis of liver and adipose tissue revealed that medium chain acids were not deposited in the tissues, thus suggesting that they had been completely catabolised.

Overall, these studies demonstrate that tube-feeding a single nutrient, such as fat, abolishes the precise compensation of food intake previously seen when rats are intubated with balanced diets (Rothwell & Stock, 1978). The results of feeding coconut oil and MCT indicate that medium chain fatty acids are rapidly oxidized and produce a decreased body weight gain and a large increase in oxygen consumption. The prolonged stimulation of resting metabolic rate suggests that medium chain acids may cause some adaptive change in oxidative metabolism.

Rothwell, N. J. & Stock, M. J. (1978). *Nature, Lond.* **273**, 146.

The efficiency of energy-reduced diets in the treatment of obesity by dietitians. By C. A. COOPER and A. E. DE LOOY, *School of Health and Applied Sciences, Leeds Polytechnic, LS1 2HE* and M. A. CONRY, *Dietetic Department, Leeds General Infirmary, Leeds*

Obesity has been widely recognized by the medical profession not only as the commonest nutritional problem in Great Britain today, but also as having serious consequences on health. Little or no objective analysis has been carried out on the effectiveness of dietitians using energy-reduced diets in the treatment of obesity. Dietitians spend a great deal of their time teaching overweight and obese patients methods of weight reduction with little feedback on their success.

Our aim was to examine the many variables that might influence weight loss in these patients, and to establish the results achieved by dietitians in the treatment of overweight and obese patients in a hospital dietetic department. Finally, to note any change in weight several years after the patient had stopped attending the dietetic department.

A retrospective study was carried out on a sample of obese patients discharged from the Dietetic Department at Leeds General Infirmary between 1970 and 1975. Using patients dietetic record cards, a random sample of 1000 was taken from the total 6000 record cards for analysis. Also, 100 of these patients were followed up using a questionnaire to establish their present weight.

Information about the patients in the sample undergoing dietetic care is given in the table below.

Characteristics of the sample population under study

(Numbers given in parentheses are percentages)

		No. of patients	
Female		613	(63)
Male		361	(37)
	Total	974	(100)
Age: < 40 years		228	(24)
Age: > 40 years		746	(76)
	Total	974	(100)
Occupation			
Household duties		274	(28)
Manual workers		136	(14)
Retired workers		137	(14)
Others (office, engineering, administrative)		427	(44)
	Total	974	(100)

Preliminary analysis reveals that 10% of the sample achieved their ideal weight. The mean duration of attendance was 24 weeks. 75% of the sample discharged themselves, the majority after the first week.

Results will be discussed and further analyses presented.

Grateful thanks to J. A. Webster and staff of the Maths and Computing Department at Leeds Polytechnic for their help with the computer analysis.

The effects of exercise and exercise training on dietary induced thermogenesis. By M. GLEESON, J. F. BROWN and J. J. WARING, *Biology Division, Preston Polytechnic, Preston PR1 2TQ* and M. J. STOCK, *Department of Physiology, Queen Elizabeth College, London W8 7AH*

It is well known that biochemical adaptation to exercise training occurs (Holloszy & Booth, 1976) and there are several reports (e.g. Miller *et al.* 1967) indicating that exercise increases the thermogenic effect of a meal. However, the effect of exercise training and type of diet on dietary induced thermogenesis appears not to have been studied, and is the subject of the work reported here.

Forty male Wistar rats weighing 200 g at the start of the experiment were fed balanced corn-oil based or corn-starch based diets with or without daily swimming exercise, which for the exercise groups, was for 1 h/d in a tank containing water at 34°.

Energy expenditure in the fasted and fed states and during rest or exercise, was measured in a respiration chamber. The chamber was filled with water at 34° for the exercise periods which immediately followed feeding or overnight fast. Energy expended was calculated from the oxygen consumption and respiratory quotient, in the case of resting metabolism and from oxygen consumption only, for exercise metabolism. Thermogenesis was calculated as:

$$\frac{\text{Increases in energy expenditure when fed}}{\text{Fasting resting energy expenditure}} \times 100$$

Table 1. *Energy expenditure of fed and fasted rats at rest and during exercise*

(Results are mean values with their standard errors)

	Resting			Exercising		
	Fasted (kJ/100 g per h)	Fed (kJ/100 g per h)	Thermogenesis (%)	Fasted (kJ/100 g per h)	Fed (kJ/100 g per h)	Thermogenesis (%)
Starch sedentary	2·26 (0·04)	2·62 (0·08)	16 (3)	5·33 (0·23)	5·72 (0·33)	17
Starch exercise	2·24 (0·06)	2·82 (0·07)	*26 (5)	4·88 (0·37)	5·38 (0·20)	22
Corn-oil sedentary	2·28 (0·06)	2·64 (0·09)	16 (2)	4·99 (0·42)	5·30 (0·41)	14
Corn-oil exercise	2·23 (0·04)	2·55 (0·03)	15 (2)	4·96 (0·24)	5·27 (0·33)	14

*Significantly different from other means: $P<0.05$.

Neither exercise training nor diet affected resting metabolism but on the starch diet, exercise training significantly increased dietary thermogenesis. A similar, though non-significant result, was obtained during exercise.

There was no evidence to indicate that exercise *per se* potentiates dietary thermogenesis in normal meal-fed rats.

Holloszy, J. O. & Booth, F. W. (1976). *Ann. Rev. Physiol.* **38**, 273.
Miller, D. S., Mumford, P. & Stock, M. J. (1967). *Am. J. clin. Nutr.* **20**, 1223.

Phosphorus availability from *n*-paraffin-grown yeast. By N. B. LOGAN and R. J. NEALE, *Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD*

The phosphorus content of single cell protein is high, varying from 16 to 24 g/kg and most workers report that phosphorus availability to chicks is complete (Yoshida & Hoshii, 1977). Oguntona *et al.* (1977), however, reported that phosphorus from *n*-paraffin-grown yeasts is not highly available to chicks since supplementation of high yeast diets with inorganic phosphorus improved phosphorus retention and tibia ash. We have measured the levels of phytate phosphorus in samples of *n*-paraffin-grown yeast and these vary considerably from 0·3 to 3·3 g/kg yeast (1·8 to 18% total phosphorus).

Because of the possibility that it is the yeast phytate phosphorus that is poorly available to chicks we have reinvestigated total phosphorus availability from *n*-paraffin-grown yeast included at different levels in purified diets fed to chicks by measurement of toe and tibia ash. The control diets (A, B, C and D) were based on free amino acids, sucrose and starch and contained 1, 3, 4 and 6 g/kg phosphorus as dicalcium phosphate. The test diets (E, F and G) contained 3 levels of *n*-paraffin-grown yeast (124, 185 and 309 g/kg) to provide a total of 3, 4 and 6 g/kg phosphorus. Calcium, crude protein and metabolisable energy of all diets was maintained constant.

All diets were fed to four groups of three male Cobb chicks for 10 d. The toe ash, measured as percentage dry weight was 8·06, 11·14, 13·47 and 14·87 for diets A, B, C and D and 8·81, 10·33 and 12·85 for diets E, F and G. Plotting the toe ash against the amount of additional dietary phosphorus for both control and test diets enabled phosphorus availability of the yeast to be calculated as the ratio of the slope of the line for the yeast diets divided by the slope of the line for dicalcium phosphate diets. The phosphorus availability calculated as such was 58·2%±13·7 (95% confidence limits). Since this sample of yeast contained only 0·3 g phytate phosphorus/kg yeast (1·8% total phosphorus) we are led to conclude that the low phosphorus availability from yeast is not due to its high content of phytate phosphorus.

We acknowledge the support of BP Proteins in carrying out this work.

Oguntona, T., Neale, R. J. & Lewis, D. (1977). *Proc. Nutr. Soc.* **36**, 11A.
Yoshida, M. & Hoshii, H. (1977). *Japan Poultry Sci.* **14**, 33.

The effect of an antibacterial growth promoter on the levels of alkaline phosphatase (*EC* 3.1.3.1) in the intestinal mucosa of chicks. By P. H. RIPLEY and D. J. BROWN, *The Boots Company Ltd, Thurgarton Research Station, Nottingham NG14 7GX*

Certain physiological differences between the gut wall of germ-free and conventionally-reared animals may account for the increased weight gain and food utilization observed in germ-free animals. In particular, gut wall thickness is reduced and villus length and profile are altered in the germ-free animal (Cook & Bird, 1973).

In addition, Yolton *et al.* (1977) found higher alkaline phosphatase levels in the intestinal mucosa of germ-free as compared to conventionally-reared mice, and that contamination of germ-free mice with certain bacteria reduced mucosal alkaline phosphatase levels.

A series of trials were conducted to ascertain whether an antibacterial growth-promoter might similarly increase mucosal alkaline phosphatase levels in the chick. Groups of 20 d old chicks were reared, five to a cage, in a room maintained at 29°. Water and food were supplied *ad lib.* In each trial one group received food medicated with Zinc Bacitracin (Apothekernes Laboratorium) at approximately 20 mg/kg diet and the other received an unmedicated diet. On days 4 and 7, 10 chicks from each group were killed, and the duodenum and small intestine removed and homogenized for alkaline phosphatase determination. Table 1 summarises the duodenal and intestinal levels of alkaline phosphatase obtained in one such trial.

	Birds killed at 4 d of age		Birds killed at 7 d of age	
	Treated	Control	Treated	Control
No. of Birds/group	10	10	10	10
Mean weight gain (g)	16·5	14·0	33·7*	24·05*
Duodenal AP level (I.U/g)	103·2**	42·9	75·3	53·4
Intestinal AP level	76·2**	29·7	43·45	34·75

Significance of difference from control: $^{*}P<0.05$, $^{**}P<0.01$.

Alkaline phosphatases are present in very high levels in the duodenal mucosa of chicks, and are thought to play a role in the absorption from the gut of certain nutrients (Moog & Glazier, 1972).

These tests show that certain antibacterial growth-promoters increase alkaline phosphatase levels in the intestinal mucosa of chicks, and that this effect precedes that on weight gain.

Cook, R. H. & Bird, F. H., (1973). *Poult. Sci.* **52**, 2276.
Moog, F. & Glazier, H. S., (1972). *Comp. Biochem. Physiol.* **42A**, 321.
Yolton, D. P., Stanley, C. & Savage, D. C., (1971). *Infection and Immunity* **3**, 768.

Urinary excretion of 3-methyl histidine in cattle as a measure of muscle protein degradation. By C. I. HARRIS and G. MILNE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

In view of the value of a non-destructive measure of muscle protein degradation in cattle, the kinetics of urinary excretion of 3-methyl histidine have been examined in four non-lactating animals weighing 200–625 kg. Urinary recovery of radioactivity was monitored after intravenous administration of 100 μCi of [3-^{14}C-Me]methyl histidine in sterile saline. Recoveries of radioactivity in urine were normally greater than 90% after 6 d and the proportion of excreted radioactivity associated with 3-methyl histidine (see table) was very similar to that occurring in the injected preparation, suggesting that partial metabolism of 3-methyl histidine does not occur in cattle.

Animal No.	Wt (kg)	6 d urinary recovery of radioactivity (% injected dose)	Proportion of radioactivity as 3-Me-histidine in daily urine collections (%)†	Non-protein bound 3-Me-histidine levels in muscle (n mol/g) Free	Total
439	204	90·7*	85·4–94·3 (4)		
	239	86·0	86·2–91·2 (4)	—	141·1
440	212	93·7	—		
	260	93·8	82·2–89·7 (4)	9·6	63·9
200	370	92·8	86·2–89·1 (3)		
	380	93·6	83·6–87·9 (2)	—	87·8
981	580	91·7	—		
	623	99·0	85·9–95·7 (4)	3·9	103·7

*5 day recovery.
†Period of collection in parentheses (d).

The relatively rapid elimination of the dose of 3-[^{14}C-Me]methyl histidine implies a small body pool of non-protein bound 3-methyl histidine and suggests the non-protein, peptide-bound form of 3-methyl histidine, identified in sheep and pigs (Milne & Harris, 1978), is essentially absent in cattle. The low levels of free 3-methyl histidine in tissues have been confirmed by analysis of the perchloric acid soluble extract of blood and m. longissimus dorsi. Acid hydrolysis of these samples resulted in increases in 3-methyl histidine (see table) to values less than those found in sheep and pigs (Milne & Harris, 1978).

The above results suggest the urinary excretion of 3-methyl histidine to be a valid index of muscle protein degradation in cattle, assuming that most of the 3-methyl histidine originates from muscle protein.

Milne, G. & Harris, C. I. (1978). *Proc. Nutr. Soc.* 37, 18A.

Protein turnover in rats following a short period of overfeeding.

By Z. GLICK, P. J. GARLICK and M. A. MCNURLAN, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT*

Although much is known about changes in the rates of protein synthesis in individual tissues in rats fed low protein diets or completely starved (Waterlow & Stephen, 1968, Garlick *et al.* 1975), little is known about changes with excessive intakes of nutrients. The importance of studying the latter, in addition to contributing to a better understanding of protein metabolism, lies in the possibility that an increased rate of protein turnover during excessive eating might constitute a metabolic pathway for dietary thermogenesis. This possibility seemed plausible since protein turnover contributes substantially to the basal energy expenditure (Waterlow, 1975) and is positively correlated with BMR in humans (Young *et al.* 1975) and other mammalian species (Waterlow *et al.* 1978).

Female rats weight 200–300 g were used, and their individual *ad lib.* daily intakes of a liquid diet (Vivonex, standard 5·5 kJ/ml (1·3 kcal/ml), 8·2 NDPEnergy%) were established. The rats then received their entire intakes via continuous gastric infusions through permanently implanted cannulas. The 'normal fed' rats received their individual *ad lib.* intakes only, and the 'overfed' rats received a 30% excess of the same diet over their normal intakes.

After four days of intragastric feeding, the rats were infused with [^{14}C]-tyrosine for six hours and the rates of protein synthesis determined in liver and muscle (Garlick *et al.* 1975).

The fractional rate of protein synthesis in muscle (gastrocnemius) was 5%/d in both the control and the overfed groups. This agrees with previously reported values in adult rats maintained on a cubed diet (Millward *et al.* 1975). In the liver the fractional rate of protein synthesis was lower in the overfed group by about 25%. The RNA:protein was also lower in livers from the overfed groups. It thus appears that an increase in protein synthesis in liver and muscle during overfeeding does not serve as a metabolic outlet for dietary thermogenesis under our specified experimental conditions.

Garlick, P. J., Millward, D. J., James, W. P. T. & Waterlow, J. C. (1975). *Biochim. biophys. Acta.* **414**, 71.
Millward, D. J., Garlick, P. J., Stewart, R. J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975). *Biochem. J.* **150**, 235.
Waterlow, J. C. (1975). *Nature, Lond.* **253**, 157.
Waterlow, J. C. & Stephen, J. M. L. (1968). *Clin. Sci.* **35**, 287.
Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978). *Protein Turnover in Mammalian Tissues and in Whole Body.* Amsterdam: Elsevier Press.
Young, V. N., Steffee, W. P., Pencharz, P. B., Winterer, J. C. & Schrimshaw, N. S. (1975). *Nature, Lond.* **253**, 192.

Selenium deficiency in Friesian steers. By J. R. ARTHUR, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Yearling cattle receiving diets deficient in selenium and vitamin E may develop a diffuse myopathy known as paralytic myoglobinuria (Allen *et al.* 1975). Here an attempt to induce myopathy in cattle fed a semi-synthetic diet, containing small amounts of selenium and vitamin E, is described.

Four Friesian steers (3 months of age) were offered a diet based on oat husks, Torula yeast and urea, containing 0·01 mg/kg Se (low Se group). A further four steers were maintained on the same basal diet supplemented with 0·1 mg/kg Se as sodium selenite (control group). Both diets were low in vitamin E (1·79 mg α tocopherol/kg DM).

The whole blood Se content and glutathione peroxidase activity was monitored in both groups of steers. Results after 33 and 45 weeks of experiment are given in Table 1.

Table 1. *The glutathione peroxidase (GSHpx) activity and selenium content of whole blood obtained from steers receiving selenium supplemented and unsupplemented diets*

(Results are mean values with standard errors)

	GSHpx units/ml whole blood		Se mg/litre whole blood	
Period (weeks)	33	45	33	45
Low Se group	nd	nd	0·003 0·001	0·008 0·001
Control group	28·87* 2·13	24·43* 1·55	0·107* 0·003	0·105* 0·008

nd, not detected.
*Significantly different from unsupplemented group: $P<0.001$.

After 33 and 45 weeks the plasma vitamin E of both groups was <10 μg/100 ml, the minimum detectable by the colorimetric assay used (modification of Hashim & Schuttringer, 1966). However, despite the low selenium and vitamin E status of the animals in the unsupplemented diet they displayed no overt signs of nutritional muscular dystrophy. Although plasma activities of creatine phosphokinase were elevated in the low Se animals in comparison to the control animals e.g. (291±119 *v.* 16·03±2·96 units/ml plasma, week 33), they were well below those encountered in clinical cases of nutritional myopathy and indicated only mild damage. The results suggest that other factors additional to a low Se and vitamin E status may be needed to precipitate nutritional myopathy in cattle.

Allen, W. M., Bradley, R., Barrett, S., Parr, W. H., Swannack, K., Barton, C. R. Q. & Macphee, A. (1975). *Br. vet. J.* 131, 292.
Hashim, S. A. & Schuttringer, G. R. (1966). *Am. J. clin. Nutrition* 19, 137.

Defective leucocyte function in selenium deficient cattle. By R. BOYNE and J. R. ARTHUR, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

During the ingestion of particles by neutrophils a ten to twenty fold increase in cellular oxygen consumption occurs (review Roos, 1977). This is accompanied by the production of large amounts of hydrogen peroxide which may induce peroxidation of ingested and endogenous lipids (Stossel *et al.* 1974). These peroxides are potentially injurious to the cell and may be removed by the action of glutathione peroxidase (GSHpx) (*EC* 1.11.1.9). Low neutrophil GSHpx activity has been associated with defective microbicidal activity of neutrophils from selenium deficient rats (Serfass & Ganther, 1975). Here we report the effect of selenium deficiency on neutrophil function in cattle.

Neutrophils were prepared from whole blood (Carlson & Kaneko, 1973) obtained from Friesian steers offered a Se deficient diet and a control group of steers fed the same diet supplemented with 0·1 mg/kg Se as Sodium selenite.

The ability of these neutrophils in suspension to ingest and kill *C. albicans* was investigated the latter being monitored by the failure of dead *C. albicans* cells to exclude methylene blue.

Table 1. *The effect of Se deficiency on neutrophil function*

(Results are mean values with SEM, No. of determinations in parentheses)

	C. albicans ingested/ 100 neutrophils	% Neutrophils killing ingested *C. albicans*
Se deficient	263 ± 20 (3)	17 ± 3 (3)
Se supplemented	268 ± 16 (3)	52 ± 9 (3)
Significance of difference between groups Students' t test	Not significant	$P<0.025$

Selenium deficiency did not impair the ability of neutrophils to ingest *C. albicans* cells. However, the ability of neutrophils to kill *C. albicans*, once ingested, was greatly impaired.

Neutrophils from Se deficient steers had no detectable GSHpx activity, hence the destruction of hydrogen peroxide and lipid hydroperoxides would probably be impaired in comparison to the neutrophils from the selenium supplemented controls. Neutrophils from control steers had similar glutathione peroxidase activities to red blood cells from animals of adequate selenium status.

The inability to destroy toxic peroxides may result in peroxidative damage to neutrophil lipids and proteins and thus induce defects in microbicidal activity. These effects would be especially apparent when the neutrophil is challenged by a foreign particle and in consequence produces more hydrogen peroxide.

Carlson, G. P. & Kaneko, J. J. (1973). *Proc. Soc. exp. Biol. Med.* **142**, 853.
Roos, D. (1977). *Trends in Bioch. Sci.* **2**, 61.
Serfass, R. E. & Ganther, H. E. (1975). *Nature, Lond.* **255**, 640.
Stossel, T. P., Mason, R. J. & Smith, A. L. (1974). *J. clin. Invest.* **54**, 638.

Vegan diet and lifestyle; A preliminary study by postal questionnaire.

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Although a vegan diet can provide an excellent supply of nutrients (Ellis & Mumford, 1967) there are few results on the range of dietary intakes in vegan populations based on formal sampling procedures. Similarly, although vegans differ in many respects beside their diet from the general omniverous population (McKenzie, 1971) their demographic and other characteristics have not been well defined.

A random sample of members of the Vegan Society were contacted by post and asked to complete a 4 d diet diary and a questionnaire on their views. Sixty-three members replied and 53 completed the questionnaire.

Thirty-four (64%) of the respondents were female and 19 (36%) were male. A high proportion were either in the 25–34 year old group or more than 65 years old; the majority were in social classes I or II. The median time for which the respondents had been vegan was 5 years (range 1·5 to 34 years). All except three had been vegetarian before becoming vegan.

Nearly all respondents (83%) ranked concern about cruelty and exploitation of animals as the most important reason for adopting veganism while concern for their health and concern about world food shortages were ranked second and third in importance. Twenty-seven (51%) of the respondents supplemented their diet with vitamin or mineral tablets. Examination of the forty-two diaries which were completed in sufficient detail suggested that 10 subjects had energy intakes less than 6·5 MJ/d, 7 had vitamin B12 intakes less than 0·5 μg/d and 10 had vitamin D intakes less than 1 μg/d.

This study suggests that the demographic characteristics of a vegan population are different from that of the general population and that there are a small minority of vegans who are consuming diets which are deficient in certain nutrients.

We thank the secretary and members of the Vegan Society for their wholehearted co-operation in this study.

Ellis, F. R. & Mumford, P. (1967). *Proc. Nutr. Soc.* **26**, 205.
McKenzie, J. (1971). *Pl. Fds. Hum. Nutr.* **2**, 79.

The hydrolysis of dipeptides by mucosa from different regions of the alimentary tract of the Brown Trout (*Salmo trutta*). By R. ASH, *School of Applied Biology, University of Bradford, Bradford, West Yorkshire BD7 1DP*

The trout in its natural environment is generally considered to be carnivorous, a point that is reflected in the high protein content (approximately 50%) of commercially available trout feeding pellets. However, scanty information is presently available concerning the final stages of protein digestion and absorption in this species. This study was designed to investigate the potential of various sections of the trout gut to hydrolyse three specific dipeptides, and to further provide initial information on the localization of this activity in, or upon, the epithelial absorptive (mucosal) cell.

Brown Trout were taken from a local reservoir during early May. The fish ranged in weight from 350–500 g and were of length 32–37 cm. Casual examination of stomach contents revealed that in all cases the diet consisted predominantly of Caddis larvae. The alimentary tract was removed immediately and divided into five readily distinguishable sections (see Table), each of which was opened and washed prior to storage in solid CO_2 for transport to the laboratory. Tissue preparation and measurement of hydrolytic capacity with respect to the various substrates (see Table), were essentially as described in the two step assay procedure of Nicholson & Kim (1975). Enzyme activity is expressed in μmol substrate hydrolysed/min per g wet weight at 15°.

Although optimum conditions for each peptide were used (with the possible exception of cation requirements), the method of assay does not ensure that only one enzyme is being measured in the crude mucosal extract. The results therefore represent a measure only of the distribution of the ability of the intestine to hydrolyse peptides and not the distribution of particular enzymes.

p-Hydroxymercuribenzoate (PMB) was used as a specific inhibitor of cytoplasmic dipeptidases in order to assess the relative contribution of cytoplasmic and brush border peptide hydrolase activities to the total activity observed (Nicholson & Kim, 1975).

Table 1. *Hydrolytic activity of various sections of the Trout gut with respect to the dipeptides glycyl-L-phenylalanine (GP); L-phenylalanylglycine (PG); and glycyl-L-leucine (GL)*

(Mean values with standard errors for four fish)

	Substrates								
	GL*		+PMB	PG		+PMB	GP		+PMB
Gut Section	mean	SEM	(% decrease)	mean	SEM	(% decrease)	mean	SEM	(% decrease)
Stomach	9·33	0·17	—	9·67	0·23	—	6·00	0·29	—
Ceca	277	8·6	84	60	1·8	36	243	6·4	92
Cecal intestine†	187	4·2	85	37	0·9	37	102	2·2	88
Post cecal intestine	189	4·0	83	30	1·1	37	92	4·2	87
Large intestine	170‡		77	45	2·0	31	106	1·7	92

*Activity is expressed as μmol-substrate hydrolysed/min per g wet wt at 15°.
†Region of intestine bearing ceca.
‡Only two fish.

Nicholson, J. A. & Kim, Y. S. (1975). *Analyt. Biochem.* **63**, 110–117.

The role of catecholamines and thyroid hormones in the metabolic response to semistarvation. By R. T. JUNG, P. S. SHETTY, M. BARRAND, B. A. CALLINGHAM and W. P. T. JAMES, *Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Cambridge and Department of Pharmacology, University of Cambridge, Cambridge*

Eleven obese women, of weights ranging from 80 to 130 kg (98·35±4·01 kg; mean±SEM) and aged 37·4±4·1 years were investigated in a metabolic ward. Initially the subjects were equilibrated for 5–9 d on a weight maintenance diet (HED) calculated on the basis of 167 kJ (40 kcal) /kg desirable weight. They were then put on a low energy diet (LED) providing 38·5 kJ (9·2 kcal) /kg desirable weight for 14 d. The diets had the same protein (80 g), fat (10 g), mineral and vitamin content: the reduction in energy intake was achieved by decreasing only the carbohydrate intake. Sodium intake was kept constant at 52 mmol/d.

The resting metabolic rate (RMR) was measured by the ventilated hood technique on three occasions while on the HED and then at 3 d intervals during the LED. The RMR did not fall till after the 4th day on the LED, the fall reaching significance by the 8th day. Plasma noradrenaline measured by radio-enzymatic assay declined from 0·17±0·04 ng/ml on the HED to 0·07±0·02 ng/ml ($P<0·05$) by the 12th day on the LED. No significant change in plasma adrenaline was found.

While on the HED, the daily urinary excretion of hydroxy-methoxy-mandelic acid (HMMA) was constant (22·6±1·8 μmol/d). On the LED, HMMA excretion decreased on the second day reaching significance by the third day (16·0±1·8 μmol/d, $P<0·02$) and remained low throughout the period of semistarvation.

Serum thyroxine remained unchanged on the LED but serum tri-iodothyronine (T_3) decreased and reverse tri-iodothyronine (rT_3) increased within 72 h of the LED.

Recent evidence in experimental animals suggests a decreased turnover of noradrenaline on starvation (Young & Landsberg, 1977). Our results would suggest that a similar phenomena occurs in humans on energy restriction. It would appear that both catecholamines and T_3 are implicated in the metabolic adaptation to semistarvation.

Young, J. B. & Landsberg, L. (1977). *Science* 196, 1473.

The effect of catecholamine replacement on the metabolic response to semistarvation. By P. S. SHETTY, R. T. JUNG, M. BARRAND, B. A. CALLINGHAM and W. P. T. JAMES, *Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Cambridge and Department of Pharmacology, University of Cambridge, Cambridge*

Twelve obese patients of weights ranging from 88·3 to 129·9 kg (99·98± 3·61 kg, mean±SEM) and of mean age 40·9±4·9 years were investigated in a metabolic ward for a total duration of 5 weeks. Initially they were equilibrated for a period of 5–7 d on a weight maintenance diet (HED) calculated to provide 167 kJ (40 kcal) of energy/kg desirable weight. They were then put on a low energy diet (LED) having only 38·5 kJ (9·2 kcal) energy/kg desirable weight for a further period of 28 d. Both diets were identical in their content of protein (80 g) and fat; the energy restriction was achieved by decreasing the carbohydrate intake only. Sodium intake was kept constant at 52 mmol/d. Resting metabolic rate (RMR) was measured by the ventilated hood technique every third day during the duration of the study.

To investigate whether a fall in catecholamine turnover, associated with a LED, is an important factor contributing to the decline in the RMR on semistarvation a precursor of catecholamines, L-Dopa, was given to five of the subjects. The drug was started on day 1 of the LED and the dose increased gradually to 4 g by day 8. L-Dopa prevented the usual decline in RMR on semistarvation. Serum Thyroxine (T_4) remained unchanged with L-Dopa but serum tri-iodothyronine (T_3) decreased in a similar fashion to that found on LED alone. Whereas in semistarvation the concentration of rT_3 rises, the addition of L-Dopa usually suppressed this response.

In order to assess whether L-Dopa could lead to a reversion of the RMR to normal once it had declined in response to a LED, a further seven subjects were given 2 g of L-Dopa on day 14 of the LED. L-Dopa prevented the expected further decline in RMR but failed to restore the RMR to the original level found on the HED. In these seven subjects plasma noradrenaline measured by radio-enzymatic assay was 0·20±0·04 ng/ml on the HED. By day 14 of LED the plasma noradrenaline had decreased to 0·09±0·03 ng/ml, and with L-Dopa it rose again to 0·16±0·03 ng/ml. Plasma adrenaline which was 0·04±0·01 ng/ml on HED, was unaltered on day 14 of LED, but increased slightly to 0·06±0·02 ng/ml with L-Dopa. Thus L-Dopa, a substrate for catecholamine synthesis, does enhance circulating levels of catecholamines. This study indicates that changes in RMR and T_3 can be dissociated by giving a precursor of the catecholamines so that RMR is maintained despite a marked fall in T_3. The mechanism for the metabolic adaptation to semistarvation may thus relate to an interplay of catecholamines and the thyroid hormones.

Interconversion of glutamate and glutamine in the placenta during development of foetal lambs. By JENNIFER M. PELL, MARJORIE K. JEACOCK and D. A. L. SHEPHERD, *Department of Physiology & Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ*

The role of glutamate and glutamine in the interorganal transport and excretion of nitrogen during development of foetal sheep is not fully elucidated although mean concentrations of these amino acids in umbilical venous and arterial blood and maternal arterial blood have been reported in acute (Smith *et al.* 1977) and chronic (Lemons *et al.* 1976) preparations. A study has been made of the concentrations of glutamate and glutamine determined by enzymic analysis of whole blood from the uterine and umbilical circulations in chronically catheterised sheep throughout the last third of gestation.

Comparison of glutamate concentrations in foetal arterial and umbilical venous blood indicated that there was no net release of glutamate from the placenta into the foetal circulation. In some animals there was a significant ($P<0.01$) placental uptake from umbilical blood. There were significant declines in the concentrations of glutamate in foetal arterial blood ($P<0.01$) and umbilical venous blood ($P<0.01$) from approximately 200 μmol/l at 110 d conceptual age to 120 μmol/l at 140 d. There was no significant difference between the concentrations of glutamate in maternal arterial and uterine venous blood and hence there was no net transfer of glutamate across the placenta. The concentration of glutamate in uterine venous blood remained constant during the last third of gestation (mean 143±3 μmol/l *n* 78).

The concentration of glutamine in umbilical venous blood did not change (mean 306±10 μmol/l *n* 38). However, the concentration in the foetal arterial circulation declined significantly ($P<0.01$) from a value similar to that in the umbilical vein at about 110 d conceptual age to a value of 220 μmol/l at 140 d. Hence, near term, there is a net release of glutamine from the placenta into the umbilical circulation. The concentrations of glutamine in maternal arterial and uterine venous blood declined significantly ($P<0.05$). No significant arterio-venous differences were observed and therefore foetal glutamine is not derived directly from the maternal circulation.

It is concluded that any glutamate taken up by the placenta from the foetal circulation could be converted into glutamine which is then released back into the umbilical vein since glutamine synthetase (*EC* 6.3.1.2) activity has been demonstrated in ovine placentae.

We wish to thank the Agricultural Research Council for supporting this work.

Lemons, J. A., Adcock, E. W., Jones, M. D., Naughton, M. A., Meschia, G. & Battaglia, F. C. (1976). *J. clin. Invest.* **58**, 1428.
Smith, R. M., Jarrett, I. G., King, R. A. & Russell, G. R. (1977). *Biol. Neonate* **31**, 305.

Maternal eating habits and the maintenance of breast feeding. By MARGARET J. WHICHELOW, BARBARA KING and SANDRA TAYLOR, *Cambridge University School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ*

Previous work indicated that an adequate milk production was associated with a greatly increased dietary energy intake by the mother (Whichelow, 1975). The present study was designed to determine whether by advising mothers, in the early stages of lactation, of their extra energy requirements, fewer of them would produce insufficient milk and wean their babies during the first three months.

Two areas in Cambridge were chosen for study. Mothers in one area (Advised) were given dietary advice within two weeks of delivery. In the other (Control) area no advice was given, but care was taken to spend the same amount of time with the mothers.

At three months the mothers were re-interviewed. Significantly fewer mothers in the Advised area had weaned their babies due to insufficient milk, than in the Control area (see table), and this was particularly marked in the Non-Manual Social Classes. There was no difference between the Advised and Control areas in the number of mothers weaning for all other reasons. Furthermore, in the Advised area the age of weaning due to insufficient milk was later (mean $8{\cdot}64 \pm 0{\cdot}77$ weeks) than in the Control area (mean $5{\cdot}76 \pm 0{\cdot}41$ weeks) and this difference was statistically significant $P < 0{\cdot}001$. There was no difference between the areas in the age of weaning for other reasons.

	Advised Group			Control Group		
		Weaned by three months due to:			Weaned by three months due to:	
Social Class	Breast Feeding at three months	Insufficient Milk	Other Reasons	Breast Feeding at three months	Insufficient Milk	Other Reasons
All	75	*14 (13%)	17 (16%)	99	37 (24%)	16 (11%)
Non-Manual	52	5 (8%)	7 (11%)	78	16 (16%)	8 (8%)
Manual	23	**9 (21%)	10 (24%)	21	21 (42%)	8 (16%)

Difference from Control Group: *$=X^2=4{\cdot}12 P<0{\cdot}05$.

An increased appetite appears to be important since 74% of successfully breast feeding mothers reported an increased appetite during lactation compared to 45% of those who had changed to artificial feeding.

The benefits of sustaining lactation in terms of weight loss and the well being of the mother, and the later introduction of solids to the baby will be discussed.

Whichelow, M. J. (1975). *Proc. Nutr. Soc.* 35, 64A.

The effect in cattle of intravenous infusions of manganous chloride upon the rate of secretion of bile and its manganese concentration. By H. W. SYMONDS, M. J. VAGG and DENISE L. MATHER *ARC Institute for Research on Animal Diseases, Compton, Newbury, Berkshire RG16 0NN*

The role of the bovine liver in controlling plasma Mn^{2+} concentrations is being studied. In each of two Guernsey steers (steer A 200 kg, steer B 250 kg) the duodenum was modified surgically to allow bile to be sampled and its flow rate measured. The volume of bile secreted during four 30 minute periods was measured, a small sample taken and the remainder returned to the duodenum. Manganese as $McCl_2$ was then infused into a jugular vein at 35 μg/min for 2·5 h in steer A and at 69 μg/min for 3·75 h in steer B. Assuming that 0·5% of dietary Mn is absorbed (Sansom *et al.* 1978) these infusion rates, maintained for 24 h are equivalent to increases in dietary Mn concentration of approximately 1100 and 2200 mg/kg Mn per d respectively. Normal dietary Mn concentrations range between 40 and 200 mg/kg. The observed effects of the infusions upon bile flow rates and Mn concentrations up to 24 h post infusion are given in Table 1.

In Steer B bile flow rate was severely reduced 24 h after the infusion and did not return to normal for 5 d. In addition its plasma bilirubin concentration increased from 1·1 to 60 mg/100 ml and its plasma GDH, γGT and SDH activities were increased.

Table 1. *Plasma and bile Mn concentrations and bile flow rate during intravenous infusions of $MnCl_2$*

	Steer A Infusion rate 35 μg/min			Steer B Infusion rate 69 μg/min		
	Control	Final*	24 h post-infusion	Control	Final*	24 h post-infusion
Plasma Mn ng/ml	9·5	16·1	9·8	—	—	—
Bile Mn ng/ml	350	1150	320	310	3300	250
Bile flow rate ml/min	8·6	8·8	10·2	11·8	11·9	1·1

*Last 30 min of infusion.

These changes, which suggest that Mn has a hepatotoxic effect, occurred after only a short period of infusion. They substantiate the observations of Sansom *et al.* (1978) where a dietary intake of 1000 mg/kg Mn per d for 7 d apparently exceeded the ability of the bovine liver to excrete Mn.

Sansom, B. F., Symonds, H. W. & Vagg, M. J. (1978). *Res. Vet. Sci.* 24, 366.