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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Four Hundred and Seventh Meeting of the Nutrition Society was held in the Physiology Lecture Theatre, Guy's Hospital Medical School, London, on Friday, 14 *December* 1984, *when the following papers were read:*

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A role for riboflavin in iron absorption? By HILARY J. POWERS, *MRC Dunn Nutrition Unit, Cambridge CB4 1XJ*

Studies have indicated that a very likely area of riboflavin involvement in iron metabolism is via a flavin-dependent oxidoreductase capable of removing Fe from ferritin (Zaman & Verwilghen, 1977; Powers *et al.* 1983). An experiment was conducted to determine whether Fe economy in the weanling and adult rat is influenced by riboflavin status via flavin-dependent mobilizing activity in the mucosal cells of the gastrointestinal tract.

Weanling and adult female Norwegian Hooded rats were given a diet deficient in riboflavin (B_2^-) for up to 35 and 49 d respectively. Weight-matched control animals received a complete diet (B_2^+). At four stages in the course of the experiment four animals from each group were killed, and mucosal scrapings from the gut collected for measurement of FMN-dependent ferritin Fe mobilizing activity. In addition hepatic ferritin Fe and erythrocyte glutathione reductase (NAD(P)H) (*EC* 1.6.4.2) activity were measured.

FMN-dependent Fe mobilizing activity was detected in mucosal homogenates and, from the 28th day of the experiment, was significantly reduced in both weanling and adult rats that were riboflavin deficient, relative to their controls (see table).

FMN-dependent oxidoreductase activity (nmol Fe/min per mg protein) in gut mucosal homogenate of weanling and adult rats

	Weanling				Adult			
Period of treatment	B_2^-		B_2^+		B_2^-		B_2^+	
(d)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14	1·99	0·70	3·42	0·94	3·44	0·68	4·66	1·38
28	0·07**	0·02	2·40	0·50	1·14***	0·25	4·89	0·47
35	0·13**	0·07	4·07	0·94	—	—	—	—
49	—	—	—	—	0·96***	0·05	4·31	0·52

Statistically significant compared with controls (*t* test): $^{**}P<0.01$, $^{***}P<0.001$.

By the 35th day, ferritin Fe in the riboflavin-deficient weanling rats was 40·7 (SEM 8·3) μg/g liver, significantly lower than the 110·1 (SEM 2·7) μg/g liver in the controls. In the adult rats riboflavin deficiency was associated with a mean fall of 51 μg hepatic ferritin Fe/g liver over 49 d, which contrasted with a substantial increase in control animals of 69 μg/g liver.

These results suggest that the mobilization of ferritin Fe from gut mucosal cells to the blood-stream may be influenced by the activity of an FMN-dependent oxidoreductase and that riboflavin deficiency may thereby limit the accumulation or maintenance of Fe stores.

Powers, H. J., Bates, C. J. & Duerden, J. M. (1983). *International Journal for Vitamin and Nutrition Research* **53**, 371–376.
Zaman, Z. & Verwilghen, R. L. (1977). *Biochemical Society Transactions* **5**, 306.

The effects of L-alanine, L-leucine, L-tryptophan and glucagon on galactose tolerance in man. By G. F. McPHATE, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

It has been found that glucose flattens the galactose tolerance curve in man (Stenstam, 1946) whether the glucose is given orally or intravenously (Williams *et al.* 1983). The object of the present study was to test the hypothesis that glucagon, by raising the blood glucose level, may mediate an increase in galactose tolerance. Three amino acids were also chosen for the study because, when given intravenously, L-leucine is insulinogenic (Knopf *et al.* 1966), L-alanine is glucagonogenic (Muller *et al.* 1971) and L-tryptophan is both insulinogenic and glucagonogenic in man (Hedo *et al.* 1977).

After a 12 h fast, eight healthy male subjects were given D-galactose (500 mg/kg body-weight (BW)) in distilled water (4 ml/kg BW), with or without one of the three amino acids (100 mg/kg BW). In further experiments, the same oral dose of D-galactose was given at the start of a 45 min infusion of physiological saline with or without glucagon given at a rate of 5 μg/min. In all experiments, blood was removed before and at 15, 30, 45, 60, 90 and 120 min after D-galactose ingestion and the serum was analysed for galactose, glucose and alanine. The area under the galactose response curve was taken as a measure of galactose tolerance.

Areas under the galactose response curves expressed as percentages of the galactose-only response

	Mean	SEM
Galactose	100	0·0
+ L-Alanine	91	19·8
+ L-Leucine	100	11·3
+ L-Tryptophan	56	5·8
+ Saline	103	14·9
+ Glucagon	31	5·5

One way analysis of variance (ANOVA).

When L-tryptophan or glucagon were co-administered with D-galactose the galactose tolerance curve was flattened ($P<0·001$). Neither L-leucine nor L-alanine, when given with D-galactose, resulted in a significant change in galactose tolerance. The failure of L-alanine to affect galactose tolerance would seem to contradict the hypothesis but there is no evidence that oral L-alanine, in contrast to intravenous L-alanine, results in glucagon release. Furthermore, with oral L-alanine the expected glycaemia following D-galactose ingestion did not occur. Unexpectedly, a rise in serum alanine level was found after ingestion of D-galactose, except in the L-tryptophan and glucagon studies. This rise suggests an inhibition of hepatic gluconeogenesis, the reverse of which occurs in the presence of glucagon. These findings support the hypothesis that glucagon mediates an increase in galactose tolerance in man.

Hedo, J. A., Villanueva, M. L. & Marco, J. (1977). *Metabolism* **26**, 1131–1134.
Knopf, R. F., Cann, J. W., Floyd, J. C., Fajons, S. S., Rull, J. A., Guntsche, E. M. & Thiffault, C. A. (1966). *Transactions of the Association of American Physicians* **79**, 315–320.
Muller, W. A., Faloona, G. R. & Unger, R. J. (1971). *Journal of Clinical Investigations* **50**, 2215–2218.
Stenstam, T. (1946). *Acta Medica Scandinavica* (Supplement) 177.
Williams, C. A., Phillips, T. & Macdonald, I. (1983). *Metabolism* **32**, 250–256.

The influence of the type of starch and of the incorporation of soluble dietary fibre in breakfast and lunch on the levels of glucose, insulin and C-peptide in plasma. By N. A. PIKAAR, M. WEDEL, W. VAN DOKKUM and R. J. J. HERMUS, *Department of Nutrition, Institute CIVO Toxicology and Nutrition, TNO, PO Box* 360, 3700 *AJ Zeist, The Netherlands*

To examine the influence of the type of starch and the effect of different types of dietary fibre on glucose absorption, a pilot study has been performed. Four healthy young male volunteers consumed one of four standardized breakfasts of 2·3 MJ consisting of 160 g bread with 20 g low fat margarine and 40 g cream cheese (protein 19 g, fat 20 g, carbohydrate 74 g). In addition, 250 ml unsweetened tea was given. The four breakfasts differed in the type of bread: white bread, wholemeal bread, white bread with incorporation of 10 g guar gum and white bread with addition of 1·3 g Propol. Propol is a purified, high polymer glucomannan, prepared by Shimidzu Chemical Industries Co. Ltd, Hiroshima, Japan; the guar gum, quality CSAA-M200, is a product of Meyhall Chemical AG, Kreuzlingen, Switzerland.

After breakfast (4 h), one of four standardized lunches of 2·9 MJ was given, consisting of 450 g potatoes, 245 g white rice, 355 g macaroni or 440 g beans respectively, each supplemented with chicken meat and salads. All lunches contained 26 g protein, 26 g fat and 88 g carbohydrates. The four different lunches were served with or without the addition of 1·3 g Propol. Heparinized blood samples were taken every 30 min. In the plasma the levels of glucose, insulin and C-peptide were determined.

In response to the breakfast the peak time for glucose (0·6 h) was significantly ($P<0·05$) shorter after consumption of brown bread than for the other types of bread (mean 1·5 h) The peak insulin concentration and the area under the insulin curve were significantly ($P<0·01$) smaller when bread containing guar gum was consumed. Bread containing guar gum taken with breakfast tended to lower these values in the afternoon as well (second meal effect).

Insulin	Bread . . . White	Brown	Guar gum	Propol
Peak concentration (μ U/ml)	67·5	55·6	38·6	70·3
Area (μ U/ml × h)	126·1	100·4	70·4	122·4

In response to lunch, the time of peak glucose concentration was significantly ($P<0·01$) shorter after consumption of potatoes (0·3 h *v.* a mean value of 1·3 h for the other lunches). Addition of Propol to the lunch resulted in lower peak glucose concentrations for potatoes and macaroni ($P<0·05$).

		Glucose peak concentration (mmol/l)			
Propol	Lunch . . .	Potatoes	Macaroni	Rice	Beans
+		5·7	5·6	5·7	5·9
−		6·5	6·3	6·1	5·5

The peak time for insulin was significantly longer after addition of Propol to the rice lunch (1·6 h with Propol, 0·3 h without Propol ($P<0·05$)).

Selection of the type of starch-rich food and the addition of soluble dietary fibre, or both, seem to be useful for patients who may benefit from moderating the rate of glucose absorption.

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Effect of a high-fibre, low-fat and low-sodium diet on white European and black West Indian type II diabetic patients with mild hypertension.

By P. J. PACY*, P. M. DODSON, J. WEBSTER* and K. G. TAYLOR, *Department of Diabetes, Dudley Road Hospital, Birmingham*

We have reported that the prevalence of hypertension in black diabetics, of Caribbean extraction, is greater than in whites of western European origin (Pacy *et al.* 1985). We have also demonstrated that an intended diet high in unrefined carbohydrate (50% daily energy) but low in sodium (60–80 mmol/d) reduced blood pressure and improved several other cardiovascular risk factors in type II diabetics with mild hypertension (Dodson *et al.* 1984). However, there may be different ethnic responses to hypotensive therapy. Blacks tend to be more sensitive to thiazide diuretics whilst less sensitive to β-blockade than whites and compliance may be less satisfactory in blacks. During the course of a study (Dodson *et al.* 1984), nineteen blacks (seven male, twelve female) were given the modified diet and the results of these have been retrospectively compared with nineteen whites (seven male, twelve female). Whites and blacks were matched for age (56·4 (SD 6·5) *v.* 54·1 (SD 6·4) years), ideal body-weight (133·6 (SD 25·9) *v.* 129·6 (SD 24·2) %), systolic pressure (178 (SD 19) *v.* 177 (SD 26) mm Hg) and diastolic pressure (96 (SD 9) *v.* 98 (SD 9) mm Hg) respectively. Dietary manipulation for 3 months reduced systolic pressure (whites 14·2 mm Hg, $P<0·01$; blacks 20·2 mm Hg, $P<0·001$) and diastolic pressure (whites 10·3 mm Hg, $P<0·001$; blacks 9·3 mm Hg, $P<0·05$) accompanied by decreased daily urine Na excretion (whites 81·2 mmol, $P<0·001$; blacks 70·1 mmol, $P<0·01$) and Na:potassium value (whites and blacks 1·1, $P<0·001$). Glycosylated haemoglobin decreased (whites 3·0%, $P<0·001$; blacks 2·0%, $P<0·01$) as did body-weight (whites and blacks 2·2 kg, $P<0·01$). Only whites had a significant reduction of serum triglyceride (0·4 mmol/l; $P<0·05$). Similar responses were observed in hyperlipidaemic diabetics although the responses were more pronounced in whites. Using a compliance scoring system based on expected changes of urinary Na excretion, Na:K value, fasting serum cholesterol, fasting serum triglyceride, body-weight and glycosylated haemoglobin (−1 for each variable that decreased; +1 if increased), sixteen whites and eighteen blacks were considered compliers (total score negative).

These results suggest such a modified diet may be an alternative to conventional hypotensive drugs in black as well as white type II diabetics with mild hypertension.

Dodson, P. M., Pacy, P. J., Bal, P., Kubicki, A. J., Fletcher, R. F. & Taylor, K. G. (1984). *Diabetologia* **27**, 522–526.

Pacy, P. J., Dodson, P. M., Beevers, M., Fletcher, R. F. & Taylor, K. G. (1985). *Diabetic Medicine* (In the Press).

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Four year follow-up treatment of essential hypertension with a high-fibre, low-fat and low-sodium dietary regimen. By P. M. DODSON[1], P. J. PACY[1*], E. V. COX[2] and K. G. TAYLOR[1], [1]*Department of Diabetes, Dudley Road Hospital, Birmingham,* and [2]*Department of Medicine, Royal Berkshire Hospital, Reading*

We have previously reported the hypotensive response to a high-fibre (40 g/d), low-fat (25% dietary energy) and low-sodium (60–80 mmol/d) dietary regimen in thirty-two patients with essential hypertension over a 3 month period. We now report the effects and compliance after a 4 year period (mean follow-up 3·9 (SD 0·58) years) in nineteen of these patients who attended for review (mean age 57 (SD 9·6) years, eighteen males and one female, ideal body-weight 116·8 (SD 12·1) %). Dietary counselling had been performed only in the first 3 months of the study.

Of the original thirty-two patients attending the Royal Berkshire Hospital, at 4 years, three patients had died (cerebrovascular disease *n* 2, myocardial infarct *n* 1), three patients had moved from the area and seven patients were lost to follow-up. As shown in the table, the nineteen patients demonstrated significant reductions in systolic and diastolic blood pressures and weight, which were maintained at 3 months and 4 years. Of importance, the number of antihypertensive tablets taken by the group was significantly ($P<0·001$) reduced at both 3 months (60%) and 4 years (49%).

Changes observed over 4 years in nineteen essential hypertensive patients on the dietary regimen

	At entry		3 months		4 years	
	Mean	SD	Mean	SD	Mean	SD
Systolic blood pressure (mm Hg)	169	19·5	150•••	13·8	148••	19·9
Diastolic blood pressure (mm Hg)	104	10·2	90•••	6·3	87•••	6·4
Weight (kg)	76·6	9·4	71·9•••	8·4	73·4•	9·7
Number of 40-mg β-blocker tablets taken per day by the group	77		16†		19†	

Paired *t* test: •$P<0·05$, ••$P<0·01$, •••$P<0·001$. Chi squared test: †$P<0·001$.

Analysis of two non-consecutive day weighed dietary histories collected at 4 years (*n* 28) demonstrated similar values of total energy (7·5 and 7·8 MJ/d), unrefined carbohydrate (50 and 47·7% energy), fat (25 and 33% energy), sodium (80 and 103·9 mmol/d) and dietary fibre (42 and 38·1 g/d) intake to the intended diet.

We conclude that the hypotensive response accompanied by reduction in weight and antihypertensive drug therapy can be maintained in the long-term with good compliance to this dietary regimen in a fair percentage of patients (59%). This regimen may therefore be of benefit in the long-term management of essential hypertension.

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Decreased protein synthesis in skin, muscle and bone after interleukin 1 production by *Escherichia coli* endotoxin in rats. By JENNIFER WAN[1], R. F. GRIMBLE[1] and M. GORE[2], *Departments of* [1]*Nutrition* and [2]*Biochemistry, Southampton University, Southampton SO9 3TU*

Interleukin 1 (IL 1) is released from macrophages in response to trauma and infection (Kampschmidt, 1984). Acute phase protein synthesis is stimulated in liver by IL 1. The extent to which IL 1 plays a part in the loss of protein from skin, muscle and bone in response to the stress is unknown.

The present study examined the effects of endogenous IL 1 on protein synthesis in liver, skin, skeletal muscle and bone. Male Wistar rats (150 (SE 7) g) were individually caged and given standard laboratory chow. Endogenous IL 1 production was stimulated in half the rats by two intraperitoneal injections of *Escherichia coli* toxin (lipopolysaccharide B); 40 and 20 μg were given at an interval of 3 d. Protein fractional synthetic rates (FSR) were measured 3 d later in liver, mixed thigh muscle, and skin from abdomen, leg and femur (Preedy *et al.* 1983). The weights of liver, pelt and tibialis muscle and the protein contents of all tissues were measured. The stimulation of endogenous IL 1 by *E. coli* toxin was verified from the characteristic fall in serum zinc (1·81 (SE 0·03) to 1·15 (SE 0·06) μg/ml, $P<0.001$), rise in corticosterone (252 (SE 19) to 351 (SE 6) ng/ml, $P<0.001$) and rise in acute phase proteins, exhibiting antiprotease activity, exemplified by a 2·2 fold increase in antichymotrypsin and 1·7 fold increase in antitrypsin. Other results are shown in the table (six rats per group).

	Weight (g)				FSR (%/d)				Protein concentration (%)			
	Control		*E. coli*		Control		*E. coli*		Control		*E. coli*	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver	9·5	0·4	8·2*	0·4	89·5	5·4	75·8*	2·2	20·5	0·3	24·2**	0·5
Muscle†	0·43	0·02	0·37*	0·02	12·9	1·2	11·1**	0·4	16·8	0·6	16·8	0·6
Bone	—	—	—	—	68·8	5·5	50·0**	3·3	6·2	0·2	4·9**	0·3
Skin (leg)	—	—	—	—	30·1	2·2	23·4*	1·2	6·8	0·5	6·8	0·3
Skin (abdomen)	—	—	—	—	37·2	3·1	25·9*	3·0	8·5	0·1	7·3*	0·3
Skin (total)	34·7	0·6	30·9*	1·5	—	—	—	—	—	—	—	—

Significantly different from control (Student's *t* test): *$P<0.05$, **$P<0.01$.
†Weight of tibialis shown, FSR and protein concentration measured in mixed thigh muscle.

In response to *E. coli* toxin injection, protein loss occurred from skeletal muscle, skin and bone. A decrease in FSR could explain this finding. The studies of Kampschmidt (1984), on starved rats, showed that IL 1 increased protein breakdown in muscle without affecting synthesis. The present study on fed animals suggests that IL 1 could reduce protein synthesis in muscle and also in other tissues. Starvation has also been shown to reduce FSR in skin, muscle and bone (Preedy *et al.* 1983). The *E. coli* group had reduced food intakes (15 (SE 2) *v.* 20 (SE 1) g/d), thus the extent to which decreased FSR are due to IL 1 cannot be assessed.

Kampschmidt, R. F. (1984). *Journal of Leukocyte Biology* **36**, 341–355.
Preedy, V. R., McNurlan, M. A. & Garlick, P. J. (1983). *British Journal of Nutrition* **49**, 517–523.

Factors limiting endurance exercise in hot and cold environments, By C. E. FENN, *School of Nutritional Sciences, Robert Gordons' Institute of Technology, Queen's Road, Aberdeen* and R. J. MAUGHAN, *Institute of Environmental and Offshore Medicine, Foresterhill, Aberdeen*

At a work load requiring 70–75% of maximum oxygen ($\dot{V}O_2$ max), depletion of muscle glycogen and hypoglycaemia may be limiting factors in endurance exercise (Bergstrom & Hultman, 1967). It is not clear whether this is true at extremes of ambient temperature. In the present study, six male subjects exercised to exhaustion on a bicycle ergometer on six occasions, 1 week apart, at a work load requiring 70% of $\dot{V}O_2$ max. On three occasions, exercise took place at an ambient temperature of 33°, 40–50% humidity and on three occasions at 2°, 100% humidity. A randomized Latin square design was employed. Immediately before exercise and at 10-min intervals throughout, subjects ingested 100 ml water or a dilute glucose electrolyte solution (Dioralyte (g/l): 40 glucose, 1·0 sodium chloride, 1·5 potassium chloride, 1·5 sodium bicarbonate; Armour Pharmaceutical Co. Ltd) or received no drink. Drinks were maintained at 20° before ingestion. Venous blood samples were drawn before exercise and at the point of exhaustion. Exercise time was not significantly affected by fluid intake, but was significantly longer ($P<0.001$) in the cold than in the hot environment (cold: no drink 72·6 (SE 9·2), water 74·5 (SE 9·6), Dioralyte 87·2 (SE 22·8); hot: 34·5 (SE 7·2), 40·4 (SE 16·2), 36·6 (SE 9·1) min). O_2 consumption was not significantly different between the exercise tests. The rate of carbohydrate oxidation, calculated from respiratory exchange ratio and O_2 consumption, was not significantly different between hot and cold conditions, nor as a result of giving water or Dioralyte. Total carbohydrate oxidation was greater in the cold ($P<0.001$). No subject was hypoglycaemic (plasma glucose concentration <2.5 mmol/l) at exhaustion. The results are in contrast to previous findings, using a similar experimental design, that Dioralyte administration during exercise at normal room temperature (21°) was effective in increasing endurance capacity (Fenn *et al.* 1983). It seems likely that fluid loss was not a limiting factor in the present study; sweat rate was low during exercise in the cold, and the exercise time in the hot room was too short to cause significant dehydration. Since subjects exercised for longer in cold conditions, utilizing a greater quantity of carbohydrate, it appears that factors other than muscle glycogen content may be limiting exercise time at high ambient temperatures, but the nature of these factors is not clear.

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Bergstrom, J. & Hultman, E. (1967). *Journal of Clinical and Laboratory Investigation* **19**, 218–228.

Fenn, C. E., Leiper, J. B., Light, I. M. & Maughan, R. J. (1983). *Journal of Physiology* **341**, 66P.

A comparison of the food habits of students from four courses. By JANET P. LOWELL, *School of Nutritional Science, Robert Gordon's Institute of Technology, Kepplestone Premises, Queen's Road, Aberdeen AB9 2PG*

Recent reports (National Advisory Committee on Nutrition Education, 1983; Department of Health and Social Security, 1984) have recommended changes in the UK diet and have pointed out that one of the ways to achieve this is by education. At Robert Gordon's Institute of Technology, nutrition is taught as part of the curriculum in three courses. These are, in descending order of number of hours devoted to nutrition in each course, Nutrition and Dietetics (ND, 183 h), Home Economics (HE, 104 h) and Hotel, Catering and Institutional Management (HCIM, 75 h). In 1979 and 1980 a preliminary study was carried out in which students in the last term of these 3-year courses (69 ND, 52 HE and 60 HCIM students) completed a questionnaire on food habits. Eighty students on a fourth course, Pharmacy (Ph), which does not include nutrition, were also surveyed to act as a control group.

In general, ND students had a higher response rate than the mean student response, for what are regarded as better food habits, while HCIM and Ph students had a lower response rate. For the poorer food habits the response rates were generally reversed.

For example, of the ND students, only 10% did not eat breakfast (cf HE 13%, Ph 30%, HCIM 43%, mean 25%, $P<0.02$) and 61% preferred bran-containing breakfast cereals (cf HE 21%, HCIM 12%, Ph 10%, mean 26%, $P<0.001$); 65% usually ate wholemeal bread (cf HE 46%, HCIM 25%, Ph 19%, mean 38%, $P<0.001$) but only 15% usually ate white bread (cf HE 31%, HCIM 52%, Ph 56%, mean 39%, $P<0.001$). 32% of the ND students usually spread polyunsaturated margarine on bread (cf Ph 30%, HE 21%, HCIM 8%, mean 24%, $P<0.01$); 80% took no sugar in tea (cf HE 71%, HCIM 63%, Ph 54%, mean 66%, $P<0.05$) and 71% took none in coffee (cf HE 65%, HCIM 53%, Ph 36%, mean 55%, $P<0.001$). However, 44% Ph students and 42% HCIM students drank cola or other soft drinks regularly (cf HE 19%, ND 12%, mean 30%, $P<0.001$) and 45% Ph students ate chips regularly (cf HCIM 35%, HE 15%, ND 8%, mean 27%, $P<0.001$).

In order to assess whether the differences in food habits are due to the course which the students follow, a longitudinal study is in progress in which students complete the questionnaire at the beginning of the first year and again at the end of the third year.

Department of Health and Social Security (1984). *Diet and Cardiovascular Disease.* Committee on medical aspects of food policy. London: H.M. Stationery Office.

National Advisory Committee on Nutrition Education (1983). *A Discussion Paper on Proposals for Nutritional Guidelines for Health Education in Britain.* London: Health Education Council.

The accuracy of the Engstrom Metabolic Computer. By I. T. CAMPBELL and S. L. SNOWDON, *University Department of Anaesthesia, Royal Liverpool Hospital, Prescot Street, PO Box 147, Liverpool L69 3BX*

There are a number of problems associated with the measurement of the oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) of ventilated patients; namely the high inspired O_2 concentrations, high humidity, high minute volumes relative to gas exchange and the pulsatile nature of the expired gas volume. Apparatus has recently become available commercially which purports to measure the O_2 consumption and CO_2 production of ventilated patients. The Engstrom Metabolic Computer (Gambro Engstrom; Bromma, Sweden) is used in conjunction with the Engstrom Erica Ventilator and a CO_2 analyser.

The accuracy of this apparatus was investigated by adding CO_2 and nitrogen to an artificial lung, attached to the ventilator, at predetermined rates via rotameters calibrated against a spirometer and bubble flow meter. $\dot{V}O_2$ and $\dot{V}CO_2$ produced by the metabolic computer were compared with those predicted from the N_2 and CO_2 flows. Accuracy was tested over the range of minute volume (8–16 litres), inspired O_2 (FIO_2) (room air 60%) and $\dot{V}O_2$ and $\dot{V}CO_2$ (150–400 ml/min) encountered most frequently in clinical practice.

The mean error for all readings of $\dot{V}O_2$ was 0·3 (SD 3·9) %, for $\dot{V}CO_2$ 0·8 (SD 2·6) % and respiratory quotient −1·2 (SD 4·5) %. In room air, mean $\dot{V}O_2$ error was 0·1 (SD 2·3) %; at FIO_2 40%, 0·9 (SD 3·2) %; at FIO_2 50%, 3·1 (SD 6·1) %; but at FIO_2 60% and above the errors were in excess of 15%. Mean error in $\dot{V}CO_2$ was less than 2% up to an FIO_2 of 80%. There was a significant negative corelation between the size of the error and $\dot{V}O_2$ (r −0·58, $P<0·001$) and $\dot{V}CO_2$ (r −0·49, $P<0·001$), the apparatus overestimating $\dot{V}O_2$ by 2·9 (SD 1·9) % at values between 150 and 200 ml/min and underestimating by 1·6 (SD 1·9) % between 350 and 400 ml/min. The corresponding figures for $\dot{V}CO_2$ were 1·5 (SD 2·1) between 150 and 200 ml/min and −1·4 (SD 4·5) % between 350 and 400 ml/min.

At an FIO_2 of 60% the errors in $\dot{V}O_2$ and $\dot{V}CO_2$ were in excess of 15%. However, the apparatus has the facility for assuming a respiratory quotient if a CO_2 analyser is not available and when this was done at an FIO_2 of 60%, putting N_2 only into the circuitry, the mean error in $\dot{V}O_2$ was −0·8 (SD 2·0) %.

Minute volume made no difference to the size of the errors and there was no significant difference between the values obtained with and without a humidifier in the ventilator circuit.

Effects of parental age and lipid metabolism in the chick embryo. By R. C. NOBLE[1], F. LONSDALE[2], K. CONNOR[1] and D. BROWN[2], [1]*Hannah Research Institute, Ayr KA6 5HL* and [2]*West of Scotland Agriculture College, Ayr KA6 5HW*

Several studies have shown that parental age has a considerable effect upon hatchability (Smith & Bohren, 1975; Shanawany, 1985). This has proved to be a particular problem in the broiler industry where hatchability of eggs laid during the first week of sexual maturity may be as low as 40%. The reduced hatchability displayed by the eggs from the very young breeders was associated with an abnormal distribution of overall embryonic weight. In view of the role of lipid metabolism during the last week of embryonic development, the possibility existed that the abnormal distribution of overall weight might be associated with a disturbance in the transfer and metabolism of yolk lipid.

The distribution and composition of total lipid in the yolk contents, yolk sac membrane and embryonic tissues of fertile eggs from 23- and 46-week-old broiler-breeder parent birds at days 13 and 19 of incubation were determined. The amount of lipid associated with the total yolk, i.e. yolk contents plus yolk sac membrane, of the embryos from the 23-week-old parents was significantly greater ($P<0.001$) than that of the embryos from the 46-week-old parents. The distribution of lipid between the yolk contents and the yolk sac membrane was such that in the embryos from the 23-week-old parents there was a very much higher proportion ($P<0.01$) of the lipid still remaining in association with the yolk contents. In the embryos from the 23-week-old parents the proportion of lipid associated with the extrahepatic tissues had not increased by the 19th day of incubation ($P<0.001$). Differences too were observed between the distribution of major lipid constituents within the yolk contents and embryonic tissues of the eggs from the two groups of parents. Most notably the level of triglycerides within the yolk sac membranes of the eggs from the 23-week-old parents at day 19 of incubation was significantly lower ($P<0.05$) and the proportion of cholesterol esters significantly higher ($P<0.001$).

The possibility exists therefore that the higher mortality of the chick embryos from very young parents is associated with a malfunction in the yolk lipid assimilation through a reduction in the ability to mobilize the stored lipid from the yolk contents into the yolk sac membrane. Access by the embryo to the major nutrient associated with development during the last week of incubation is therefore denied.

Shanawany, M. M. (1985). *British Poultry Science* (In the Press).
Smith, K. P. & Bohren, B. B. (1975). *Poultry Science* **54**, 959–963.

The relationship between fractional growth rate and fractional rate of protein synthesis within a population of growing broilers. By LINDA BRYAN and P. J. BUTTERY, *University of Nottingham School of Agriculture, Department of Applied Biochemistry and Food Science, Sutton Bonington, Nr Loughborough, Leics. LE12 5RD* and C. FISHER, *AFRC Poultry Research Centre, Roslin, Midlothian EH25 9PS*

Individual broilers, all of the same strain, kept under uniform conditions and given a uniform diet, will grow and hence deposit protein at different rates. In an experiment designed to see what causes these different growth rates, we have measured the fractional rate of protein synthesis in the liver, leg and breast tissues of 21-d-old broilers from the same population, but with different growth rates.

Male Marshall M4 broilers (*n* 200) were reared from 1-d-old until 14 d of age in heated brooders and given a standard crumb starter diet (230 g protein/kg) *ad lib.* Individual body-weights were recorded every other day and after 14 d the mean body-weight was 240·8 (SD 45·6) g. Thirty birds varying in growth rate were chosen (fractional growth rates from 0·056/d to 0·126/d) and moved into individual cages for a further week. Growth rate and feed intake were monitored each day and at the end of the week protein synthesis was measured by the large dose phenylalanine method (Garlick *et al.* 1980).

The coefficients of correlation between the fractional growth rate (FGR) and the fractional rates of protein synthesis (FSR) are given in the table.

	Correlation coefficient	Level of significance
FSR liver *v.* FGR	0·29	NS
FSR leg *v.* FGR	0·58	$P<0·01$
FSR breast *v.* FGR	0·16	NS

NS, not significant ($P>0·05$). df 27.

The significant correlation between growth and the synthesis of leg muscle protein suggests that an increase in synthesis rate is involved in any increase in accumulation of muscle protein (Garlick *et al.* 1973). The rate of protein synthesis, however, does not seem to be important in controlling the accumulation of breast muscle protein. Maruyama *et al.* (1978), using a range of diets to obtain different growth rates, also observed these differences in the action of leg and breast muscle.

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Garlick, P. J., McNurlan, M. A. & Preedy, V. R. (1980). *Biochemical Journal* **192**, 719–723.
Garlick, P. J., Millward, D. J. & James, W. P. T. (1973). *Biochemical Journal* **136**, 935–945.
Maruyama, K., Sunde, M. L. & Swick, R. W. (1978). *Biochemical Journal* **176**, 573–582.

Effect of pregnancy and lactation on muscle protein metabolism in sheep.

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The role of maternal muscle protein as a potential amino acid source in pregnancy and lactation is not well established. In sheep, loss of maternal carcass protein in pregnancy (Robinson *et al.* 1978) and decrease in weight and protein content of individual muscles in lactation (Bryant & Smith, 1982) have been described. An arterial–venous (A–V) difference technique was used to measure amino acid balance and uptake across the hind-limb of wethers (male castrate) and dry non-pregnant (NP), pregnant (P) (13±2 d pre-partum) and lactating (L) (29±2 d post-partum) ewes. Animals were fed *ad lib.* with food available throughout the period of experiment. During a constant infusion of L-[U^{14}C]threonine arterial and tarsal venous blood samples were taken at intervals, plateau specific activities being attained by 6 h. Net protein gain was estimated from A–V concentration differences (and blood flow), and protein synthesis from extraction of ^{14}C-labelled threonine. Protein degradation was estimated from the difference. Results are shown in the table.

Muscle protein turnover (%/d)

Status of sheep	Net gain			Synthesis			Degradation		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Dry non-pregnant	0·01	0·1	3	2·1	0·4	3	2·1	0·5	3
Wethers	0·1	0·1	4	1·4	0·8	4	1·2	0·7	4
Pregnant	−1·4	0·9	7	2·8	0·7	7	4·1	1·2	7
Lactating	−0·2	0·7	9	2·8	0·3	9	3·0	0·6	9

P ewes showed a net loss of hind-limb muscle, while NP and L ewes were in approximate net balance. Both P and L ewes displayed a slight increase in the rate of muscle protein synthesis. Thus in both productive groups there was a substantial increase in the rate of protein degradation. Further evidence that protein synthesis increases in pregnancy came from measurement of rates using a more established method: the ratio of specific activities of muscle free and protein-bound threonine (Waterlow & Stephen, 1968). Synthesis rate in three NP ewes was 1·3 (SE 0·5) %/d while in four P ewes it was 2·4 (SE 0·5) %/d. Pregnancy and lactation may result in increased turnover of muscle protein but the major factor responsible for loss of muscle protein in pregnancy seems to be an increased rate of degradation.

Bryant, D. T. W. & Smith, R. W. (1982). *Journal of Agricultural Science, Cambridge* **99**, 319–323.

Robinson, J. J., McDonald, I., McHattie, I. & Pennie, K. (1978). *Journal of Agricultural Science, Cambridge* **91**, 291–304.

Waterlow, J. C. & Stephen, J. M. L. (1968). *Clinical Science* **35**, 287–305.

Zinc is highly conserved in the severely Zn-deficient rat. By PAULA PEIRCE[1], M. JACKSON[2], A. TOMKINS[1] and D. J. MILLWARD[1], [1]*Nutrition Research Unit, Department of Human Nutrition, London School of Hygiene & Tropical Medicine, 4 St Pancras Way, London NW1 2PE,* and [2]*Rayne Institute, University College Hospital, University Street, London WC1*

Previous studies of zinc homeostasis in the severely Zn-deficient rat have shown that there is a considerable redistribution of Zn from bone to muscle in response to a Zn-deficient diet and this appeared to occur with very little excretion of Zn from the body (Giugliano & Millward, 1984). This was surprising since the characteristic cyclic changes in food intake result in periodic losses in body and tissue weights which should have induced commensurate losses of Zn from the tissues. It was suggested, therefore, that Zn must be highly conserved even during the catabolic phases of the body-weight cycle. We have now carried out Zn balance studies on severely Zn-deficient rats to determine directly the extent of Zn conservation.

Ten young rats (60 g) were given a severely Zn-deficient diet (1·8 μg Zn/g) based on egg albumin with or without added Zn for up to 3 weeks. This included an 8 d balance period when measurements were made of Zn losses in urine and faeces. Average daily loss of Zn in the Zn-supplemented rats was 557 μg total Zn (13 μg in urine and 549 μg in faeces). In contrast, the Zn-deficient rats lost on average only 3·3 μg (0·8 μg in urine and 2·24 μg in faeces) and there was no difference between the Zn excretion in rats in the anabolic (weight gaining) or catabolic (weight losing) phases of their body-weight cycle. These losses are very small when it is recognized that the Zn content of rat tissues is about 15 μg/g and that during the balance period, losses of body-weight of up to 11 g/d occurred. Thus it is clear than Zn excretion is almost totally suppressed in these rats. The mechanism for this is not clear but since plasma Zn is very low and the increases in plasma Zn which occur when Zn-deficient rats are losing weight are small (Giugliano & Millward, 1984), it is possible that Zn is selectively retained in the tissues during tissue catabolism.

Giugliano, R. & Millward, D. J. (1984). *British Journal of Nutrition* **52**, 545–560.

Energy utilization during insulin-induced hyperphagia in rats. By C. J. H. WOODWARD and P. W. EMERY, *Department of Nutrition, Queen Elizabeth College, Campden Hill Road, London W8 7AH*

It has long been known that repeated injections of slow-acting insulin produce hyperphagia and obesity (Mackay *et al.* 1940). Recently, however, Rothwell *et al.* (1983) have shown that insulin promotes diet-induced thermogenesis. In view of these apparently conflicting roles, we have carried out a study of energy utilization in rats receiving slow-acting insulin.

Two groups of six female Sprague Dawley rats from the QEC colony, of average initial weight 163 g, were given a semipurified diet containing (g/kg) 200 casein, 580 carbohydrate and 100 vegetable oil. One of the groups received isophane zinc insulin (Insulatard; Nordisk) by daily subcutaneous injection. The initial dose was 20 IU/kg increasing to a maximum of 50 IU/kg. After 16 d, both groups were killed for carcass analysis. Energy content was calculated factorially from the measured amounts of fat and crude protein in the animals. Data on initial body composition were obtained from a similar group of rats killed at the start of the study.

	ME intake (kJ/d)		Tissue deposition (kJ/d)		Tissue deposition (g protein/d)		Energy expenditure (kJ/d)		Energy expenditure (kJ/d per kg $BW^{0.75}$)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Insulin treated	373	8	123	11	1·00	0·06	251	12	792	27
Control	280	8	51	4	0·75	0·03	229	7	784	21
$P<$	0·001		0·001		0·01		NS		NS	

NS, not significant. BW, body-weight.

Insulin-treated rats consumed 33% more food than controls and deposited 2·4 times as much energy. The excess gain consisted mainly of fat, although there was also a significant increase in protein deposition. Energy expenditure, calculated by difference, was not significantly different between the groups. The excess energy consumed by the insulin-treated rats was used for tissue deposition with an energetic efficiency of 76%. This value is close to the established value for fat synthesis (Pullar & Webster, 1977).

It may be concluded then, under these conditions, the effect of insulin was entirely anabolic, and that the insulin-induced hyperphagia did not provoke an adaptive increase in energy expenditure.

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Mackay, E. M., Callaway, J. W. & Barnes, R. H. (1940). *Journal of Nutrition* **20**, 59–66.
Pullar, J. D. & Webster, A. J. F. (1977). *British Journal of Nutrition* **37**, 355–363.
Rothwell, N. J., Saville, M. E. & Stock, M. J. (1983). *American Journal of Physiology* **245**, E160–E165.

Effect of a cafeteria diet on the metabolism of brown adipose tissue in young and old rats. By R. FALCOU, R. ROZEN and M. APFELBAUM, *Laboratoire de Nutrition Humaine, Faculté X. Bichat,* 16 *rue H. Huchard,* 75018 *Paris, France*

It has been reported that in rats, monotonous (Levin *et al.* 1984) or cafeteria (Rothwell & Stock, 1979) high-fat diets induce an increase in brown adipose tissue (BAT) weight and in the lipolytic response to catecholamines but not in spontaneous lipolysis. Forty-eight young (2 months old) and forty old (6 months old) Wistar rats were given a cafeteria diet as described previously (Mandenoff *et al.* 1982).

	Young rats				Old rats			
	Control		Cafeteria		Control		Cafeteria	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Body-wt (g)	381·6	3·1	408·1***	4·1	524·4	6·3	570·9***	7·8
BAT wt (mg)	411·9	13·0	641·4***	18·2	441·5	24·5	781·1***	49·3
BAT protein (mg)	18·3	0·6	32·9*	1·0	11·8	0·7	22·4***	1·7
BAT DNA (μg)	265·4	12·5	356·0***	15·5	216·3	14·3	228·3	14·5
DHA binding								
K_d (nM)	1·0	0·3	1·5	0·3	2·1	0·6	2·3	0·6
B_{max}†	28·1	2·7	81·2*	19·2	26·2	3·6	17·1*	1·4
Lipolysis								
Basal‡	1·23	0·124	1·13	0·223	1·09	0·156	1·08	0·117
Isoproterenol effect§	1·25	0·105	1·30	0·251	1·21	0·146	2·92**	0·480

DHA, [^{3}H]dihydroalprenolol (β-adrenergic antagonist); K_d, dissociation constant.
*P<0·05, **P<0·01, ***P<0·001.
†B_{max} (maximal binding capacity) expressed in fmol ligand bound/mg protein.
‡Basal lipolysis expressed in μmol glycerol/g protein.
§Lipolysis stimulated by isoproterenol (5 μM). Isoproterenol effect = $\frac{\text{(stimulated–basal)}}{\text{basal}} \times 100$.

After 3 weeks, young and old cafeteria-fed rats, as compared with their respective controls, became obese with increased BAT weights. In vitro spontaneous lipolysis did not change irrespective of diet or age. β-Adrenergic-agonist-stimulated lipolysis was not significantly different in young cafeteria-fed rats but was increased in old cafeteria-fed rats. In young and old rats K_d was not significantly different but B_{max} was increased in young cafeteria-fed rats and decreased in old cafeteria-fed rats. Thus, in young rats a short-term cafeteria diet induces the occurrence of hyperplasia of BAT without modifications in lipolytic activity and, in old rats, it induces hypertrophy and an increase in the metabolic and sympathetic nervous activities in BAT.

Levin, B. E., Finnegan, M. B., Marquet, E., Triscari, J., Comai, K. & Sullivan, A. C. (1984). *American Journal of Physiology* **246**, E418–E425.
Mandenoff, A., Lenoir, T. & Apfelbaum, M. (1982). *American Journal of Physiology* **242**, R349–R351.
Rothwell, N. J. & Stock, M. J. (1979). *Nature* **281**, 31–33.

Responsiveness of epididymal adipose tissue to catecholamines and ponderal responsiveness to a cafeteria diet in rats. By A. MANDENOFF, M. C. BERTIERE, D. BETOULLE, R. FALCOU and M. APFELBAUM, *Laboratoire de Nutrition Humaine, Faculté X. Bichat,* 16 *rue H. Huchard,* 75018 *Paris, France*

We have previously reported that during cafeteria feeding, the more obese the rat became, the smaller was its catecholamine-induced oxygen consumption (Mandenoff & Apfelbaum, 1983).

Fifty male Wistar rats (200 g) were given a cafeteria diet as described previously (Mandenoff *et al.* 1982) and fourteen were given laboratory chow only (control). After 22 weeks, the fourteen leanest (LC) and the fourteen fattest (FC) cafeteria-fed rats were retained. The three groups studied were killed by decapitation and the epididymal fat pads were removed and in vitro lipolysis studied.

	Control (*n* 14)		LC (*n* 14)		FC (*n* 14)	
	Mean	SEM	Mean	SEM	Mean	SEM
Body-wt (g)	533·6	14·9	622·9	11·2	834·3	21·2
Fat pads wt (g)	13·1	1·1	23·6	1·5	36·6	1·4
Cellularity:						
Adipocyte diameter (μm)	115·6	3·6	105·4	5·1	104·3	3·4
Adipocyte number ($\times 10^6$)	16·3	2·0	30·0	0·3	52·3	0·5
Lipolysis*:						
Basal	1·23	0·076	1·28	0·073	1·57	0·086
Stimulated†	5·44	0·382	4·27	0·320	3·53	0·213
Percentage increase	348·0	25·5	246·8	35·0	128·2	11·1

*Lipolysis expressed in μmol glycerol/g fresh tissue.
†Lipolysis stimulated by isoproterenol (5 μM).

FC rats became very obese owing to an increase of white adipose tissue characterized by great hyperplasia without hypertrophy. The basal lipolysis of epididymal white adipose tissue increased only in FC rats ($P<0·001$). The stimulation of lipolysis by isoproterenol decreased in cafeteria-fed rats ($P<0·001$) and this decrease was significantly greater in FC rats than in LC rats ($P<0·001$). Thus, the effects of catecholamines on O_2 consumption and on lipolysis of epididymal adipose tissue were smaller in FC than in LC rats. The decrease of responsiveness to catecholamines could contribute to the development of obesity in this model.

Mandenoff, A. & Apfelbaum, M. (1983). *Proceedings of the Nutrition Society* **42**, 82A.
Mandenoff, A., Lenoir, T. & Apfelbaum, M. (1982). *American Journal of Physiology* **242**, R349–R351.

The effect of molybdenum on fertility in the cow. By M. PHILLIPPO, W. R. HUMPHRIES and T. ATKINSON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Previous observations (Phillippo *et al.* 1982) suggested that fertility in cattle was only impaired by a low copper status when the latter was induced by molybdenum. The following results provide additional evidence that Mo reduces fertility in cattle of low Cu status.

Fifty Hereford–Friesian heifers were given a basal barley–straw diet which, when supplemented with either 500 mg Fe or 5 mg Mo/kg, markedly reduced liver Cu retention (see table) and plasma Cu to a similar extent during the first 36 weeks of the experiment.

Any possible effect on fertility caused by different growth rates was eliminated by restricting the food intake of one group before and of all groups after the 36th week so that they grew at the same rate as the Mo-supplemented group.

During phase 1 (36–56 weeks), oestrus was synchronized, the animals inseminated and pregnancy monitored by plasma progesterone. To permit repeated observations, pregnancy was terminated at 25 d and the animals re-inseminated 40 d later. The Mo-supplemented diet significantly reduced the conception rate compared with the normal conception rates on the Fe-supplemented and control diets.

	Control		Fe		Mo		Mo		Restricted control	
Phase 1	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Liver Cu (mg/kg dry matter)	60·8	11·8	4·2	0·5	3·0	0·5	4·4	0·5	44·0	7·0
Number pregnant	13/20		11/17		2/16		4/16		12/17	
			Mo				Fe			
Phase 2										
Liver Cu (mg/kg dry matter)	68·5	12·5	4·8	0·6	3·4	0·6	4·0	0·6	43·3	11·9
Number pregnant	11/19		3/17		1/11		11/18		12/16	

In phase 2 (56–76 weeks), when Mo was substituted for Fe, the conception rate was reduced whereas it reverted to normal when Fe replaced Mo. No significant changes in liver or blood Cu accompanied these dietary changes.

These results confirm the previous observation that Mo caused infertility in cattle. The effect was not influenced by changes in body-weight since the growth rate was the same for all experimental groups. The results obtained when dietary Mo replaced Fe and vice versa suggest that the presence of Mo rather than low Cu status was the important determinant of fertility. Whether Mo influences the maternal hormonal balance, the viability of the ova produced or the uterine environment remains to be investigated.

Phillippo, M., Humphries, W. R., Bremner, I. & Young, B. W. (1982). *Proceedings of the Nutrition Society* **41**, 80A.

Dietary and endogenous nitrogen in different regions of the digestive tract of rats after a single meal of ^{15}N-labelled barley. By I. G. PARTRIDGE*, O. SIMON and H. BERGNER, *Institute of Animal Nutrition, Department of Animal Production and Veterinary Medicine, Humboldt University, Berlin, German Democratic Republic*

Bergner & Bergner (1982) attempted to measure the true digestibility of nitrogen by the isotope dilution technique, using ^{15}N-labelled rats. As an alternative to labelling the animals, in the present trial a ^{15}N-labelled diet was given. Young male Wistar rats (87±1 g) were trained for a rapid intake of barley alone, fasted for 24 h and then offered a single meal of ^{15}N-labelled barley (5·34 atom % ^{15}N excess) for 15 min. Intakes were between 1·0 and 2·5 g. The test meal also contained Cr_2O_3 (20 mg/g). Groups of five rats were killed at 0·5, 1·0, 1·5, 2·0, 2·5, 4·0, 6·0 and 8·0 h after removal of food. The contents of the stomach, three equal segments of the small intestine and the large intestine, and also faeces, were analysed for Cr_2O_3 and for N and ^{15}N abundance in both TCA soluble and insoluble fractions. The distribution patterns of Cr_2O_3 and ^{15}N along the tract were very similar. When the disappearance of ^{15}N from the contents of the small and large intestines was expressed as a proportion of the gastric outflow of ^{15}N, a disappearance rate of 0·90 was found. On the basis of isotope dilution the proportion of dietary N in digesta was calculated. The results illustrated an intensive dilution of dietary N by endogenous secretions in all regions of the digestive tract. In the distal small intestine endogenous N accounted or 0·70 of total N. It was calculated that 17 mg endogenous N were produced by the stomach within 8 h after the single meal.

The results show that using ^{15}N-labelled feedstuffs to determine N digestibility is valuable not only because it is more accurate than the classical methods, but also since its provides an insight into the dynamics of N absorption and secretion in the digestive tract.

Bergner, U. & Bergner, H. (1982). *Archiv für Tierernährung* **32**, 841–852.

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The unsuitability of the weanling rat for assessing the protein quality of duodenal digesta from lucerne (*Medicago sativa*) silage-fed sheep. By D. HEWITT, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT* and R. C. SIDDONS, *The Grassland Research Institute, Hurley, Maidenhead, Berks SL6 5LR*

Digesta flow studies in ruminants have consistently shown that formaldehyde treatment of silages increases the amount of protein entering and absorbed from the small intestine whereas production studies have been less convincing in demonstrating a beneficial effect of such treatment. A possible explanation for this apparent discrepancy is that the formaldehyde treatment may adversely affect the efficiency of utilization of the absorbed protein. To investigate this possibility, studies were undertaken using rats to evaluate the protein quality of duodenal digesta collected from sheep receiving lucerne silages which had been treated with formic acid, formic acid and formaldehyde or formic acid and glutaraldehyde. Net non-ammonia N absorption in the sheep was increased by both formaldehyde (by 31%) and by glutaraldehyde (by 71%) (Siddons *et al.* 1984).

The digesta and silage had average pH values of 2·8 and 4·2 and contained approximately 240 and 80 g ash and 180 and 160 g amino acids/kg respectively. Freeze-dried digesta or silage was incorporated at a level of 400 g/kg into rat diets composed of semi-purified ingredients and a trace element rather than a complete mineral supplement.

On all diets the rats lost weight although the loss was greater with the digesta-containing diets (mean weight loss over 2 d was 5·5 (SD 0·85) g than with the silage-containing diets (mean 0·4 (SD 1·19) g). Loss of weight after 5 d was 1·3 (SD 1·48) g with the silage-containing diets whereas rats on the digesta-containing diets had died. The results were not affected by silage treatment. Loss of weight, and death, were not prevented by supplementation of the digesta-containing diets with lysine, methionine, magnesium or margarine fat. Cause of death was not established but the most frequent observation at post-mortem was the presence of blood in the stomach and inflamed stomach walls. Kidney histology was normal.

Thus the weanling rat appeared to be unsuitable for measuring the protein quality of these silages and digesta. In contrast, Johnson *et al.* (1978) did not report any difficulty in similar tests on digesta from sheep which were fed on diets of barley and hay supplemented with soya-bean meal or isobutylidene diurea.

The authors are grateful to Mr. A. Turvey for the histological examinations.

Johnson, I. L., Thompson, A. & Armstrong, D. G. (1978). *Proceedings of the Nutrition Society* **37**, 103A.

Siddons, R. C., Arricastres, C., Gale, D. L. & Beever, D. E. (1984). *British Journal of Nutrition* **52**, 391–401.

Ruminal metabolism of lactic acid in sheep receiving a diet of sugar beet pulp and hay. By C. J. NEWBOLD, D. G. CHAMBERLAIN and A. G. WILLIAMS, *Hannah Research Institute, Ayr KA*6 5*HL*

In sheep given grass silage diets, protozoa play an important part in the metabolism of silage lactic acid in the rumen (Chamberlain *et al.* 1983) but the question remains whether protozoa are involved in lactate metabolism more generally. Of particular interest in this connection is the ruminal metabolism of endogenous lactate arising from rumen fermentation and this was examined in the experiments reported here.

In Expt (1) rumen microbial fractions were isolated by a combination of centrifugation (Chamberlain *et al.* 1983) and filtration (Williams & Yarlett, 1982) from four sheep given a diet of sugar beet pulp and hay (65:35 w/w; 920 g dry matter (DM)/d). The fractions were incubated in vitro with added D(−) and L(+) lactate and the rate of lactate disappearance measured. For both isomers the lactate-metabolizing activity was much greater in protozoa than in bacteria; mean values (mmol lactate/g protein per h) were 0·51 (SE 0·04, *n* 6, D−) and 0·69 (SE 0·070, *n* 5, L+) for bacteria and 6·71 (SE 0·91, *n* 5, D−) and 10·60 (SE 1·54, *n* 6, L+) for the protozoal fraction.

Expt (2) examined the effects of defaunation with dioctyl sodium sulphosuccinate on rumen fermentation in vivo in sheep given a diet of sugar beet pulp and hay (80:20 w/w; 960 g DM/d). The results, given in the table, show a marked increase in ruminal concentrations of lactate with no change in pH or volatile fatty acid (VFA) proportions on defaunation.

The effects of defaunation on rumen fermentation in sheep given a diet of sugar beet pulp and hay

(Mean values for three animals)

Sheep	pH	Ammonia nitrogen (mg/l)	Acetate (mmol/mol VFA)	Propionate (mmol/mol VFA)	Butyrate (mmol/mol VFA)	Lactate (mmol/l) per d	Lactate (mmol/l) 2 h after feeding
Faunated	6·3	62	645	189	142	3·4	3·7
Defaunated	6·3	11	648	197	115	8·9	13·9
SED	0·1	5*	15	35	20	0·6*	1·6*

*$P<0.05$.

The results confirm and extend the observations of Chamberlain *et al.* (1983), showing that protozoa play a central role in ruminal lactate metabolism whether the lactate is of exogenous origin, as with silage diets, or whether the lactate arises from rumen fermentation, as with the diets used here. This raises the possibility that a reduction in protozoal numbers is a major contributory factor in the development of digestive upsets arising from an accumulation of lactic acid in the rumen.

Chamberlain, D. G., Thomas, P. C. & Anderson, F. G. (1983). *Journal of Agricultural Science, Cambridge* **101**, 47–58.

Williams, A. G. & Yarlett, N. (1982). *Journal of Applied Bacteriology* **52**, 267–270.

The production performance of ewes on 'adequate'- and 'low'-sodium diets. By ISOBEL VINCENT and R. HILL, *Royal Veterinary College, Boltons Park, Hawkshead Road, Potters Bar, Herts EN6 1NB* and A. R. MICHELL and P. MOSS, *Department of Medicine, Royal Veterinary College Hawkshead, Hawkshead Lane, North Mymmes, Hatfield, Herts AL9 7TA*

The sodium content of pasture herbage can be very low, about 1 g/kg (4·3 mmol/kg) dry matter (Vincent, 1983). The effects of a diet containing this level of Na on reproductive performance and milk production in Blackface ewes were determined. The study occupied two reproductive seasons.

The experimental low-Na diet (oats, bran, wheat straw, maize gluten, mineral and vitamin supplements) plus water, provided about 6 mmol Na/d. During lactation intake increased to about 11 mmol Na/d, the difference being associated with an overall increase in feed and nutrients. The control adequate-Na diet provided about ten times these quantities of Na, giving intakes similar to those recommended by the Agricultural Research Council (1980).

The low-Na diet had remarkably little effect on the clinical health and productivity of the ewes: there was slight evidence of 'pica' in the early stage of the experiment. The numbers of lambs born alive per hundred ewes mated, for adequate-Na and low-Na ewes, were 153 and 163 respectively in year 1 and 156 and 150 in year 2. Lamb mortality was greater among those born to low-Na compared with adequate-Na ewes but numbers surviving to lambing did not differ significantly: the values were 124 for adequate-Na and 121 for low-Na ewes in year 1 and 131 and 111 respectively in year 2.

Milk yields of small groups of ewes from each treatment group, all sucking twins, were determined from increases in lamb weight during controlled sucking periods (Peart, 1968). There was no significant treatment effect on yield in either year 1 or year 2: the overall mean daily yields during weeks 2·5–7·5 of lactation were 1·70 litres for adequate-Na ewes and 1·58 litres for low-Na ewes. The Na concentrations of milk from the two groups of ewes were also similar, at about 15 mmol/l. The daily output of Na in milk during almost all the lactation period was between 20 and 25 mmol, thus the low-Na ewes were in marked negative balance during this period.

Agricultural Research Council (1980). *The Nutrient Requirements of Farm Livestock.* Slough: Commonwealth Agricultural Bureaux.

Peart, J. N. (1968). *Journal of Agricultural Science, Cambridge* **70**, 87–94.

Vincent, I. C. (1983). A study of nutritional factors influencing reproduction and growth in Blackface sheep. PhD Thesis, University of London.

Fluid and electrolyte balance during pregnancy in ewes on 'adequate'- and 'low'-sodium diets. By A. R. MICHELL[1], P. MOSS[1], R. HILL[2] and ISOBEL VINCENT[2], [1]*Department of Medicine, Royal Veterinary College Hawkshead, Hawkshead Lane, North Mymmes, Hatfield, Herts AL9 7TA* and [2]*Royal Veterinary College, Boltons Park, Hawkshead Road, Potters Bar, Herts EN6 1NB*

Late pregnancy often causes mild oedema in humans and animals and mild hypertension is an important complication in women. Changes in the distribution or excretion of sodium are probably involved. Pregnancy toxaemia of sheep, although synonymous, is basically different, with acetonaemia the main problem. High-Na silage causes persistent udder oedema in dairy cattle (Jones *et al.* 1984). However, in most species information on Na metabolism during pregnancy and lactation is too scanty to permit sound estimates of requirement or excessive intake; indeed Na balance has not been studied in pregnant sheep (Aitken, 1976).

Fluid and electrolyte balances were examined during pregnancy and lactation in Blackface ewes kept in metabolism cages on adequate (H) or minimum practicable (L) Na intakes (approximately 1·0 and 0·1 mmol/kg body-weight respectively). The diets began 6 months before mating. Controls had the same diets but remained unmated.

Mean water intake rose by 112 (SE 36) ml/d ($P<0·05$) by day 90 in H sheep, a time of renal water retention; subsequently the rise continued until it was accompanied by polyuria and increased faecal water loss. L sheep behaved similarly but without the early retention. Aldosterone secretion rose during pregnancy solely in L ewes, suggesting a response to electrolyte balance rather than the endocrine changes of pregnancy. Aldosterone remained undetectable in H sheep, indicating that 1 mmol/kg per d is actually a liberal excess of Na, even during pregnancy. Nevertheless, their renal Na excretion fell by 12·1 (SE 1·8) mmol/d ($P<0·01$). Renal and faecal Na excretion fell to about 0·06 mmol/kg per d in L sheep, regardless of pregnancy; obligatory loss could well be less. Interestingly, Na excretion was lowest (2·9 mmol/d) in sheep which initially excreted Na predominantly in faeces; their urinary and faecal Na outputs both fell below those of sheep excreting Na predominantly in urine. These results suggest that Na requirement is far below the accepted levels. Our sheep were able to sustain pregnancy on 7 mmol/d and their obligatory losses were 3 mmol/d; neither is necessarily a minimum but both are much less than the supposed maintenance requirement for a 65 kg sheep (80 mmol/d), let alone that for reproduction. Other work suggests that Na requirement in humans and other mammals has been similarly exaggerated (Michell, 1984, 1985).

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Aitken, F. C. (1976). *Na and K in the Nutrition of Mammals*, p. 152. Farnham Royal: Commonwealth Agricultural Bureaux.
Jones, T. O., Knight, R. & Evans, R. K. (1984). *Veterinary Record* **115**, 218–219.
Michell, A. R. (1984). *Perspectives in Biology and Medicine* **27**, 221–237.
Michell, A. R. (1985). *Veterinary Record* (In the Press).

The effect of yoghurt on performance and the gut microflora of baby pigs.

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To assess the value of yoghurt as a food for young animals, pairs of Large White baby pigs of similar weight were weaned at 2 d of age and allocated to treatments of either yoghurt (Y) or the base milk (BM) from which it was prepared. The piglets were kept on these diets for 2 weeks in such a way that, within each pair, feed intakes were approximately equal. During this period, they were weighed every other day and, at the end of this time, the piglets were killed. The gut contents were examined to provide counts of lactobacilli and coliform bacteria.

The results from all trials were combined to achieve a total of twenty-four piglets on each treatment. Analysis of variance was carried out for the weight gain data and was adjusted by covariance for differences in feed intake. Taking feed intake into account, weight gains were 36% higher for the piglets fed on BM ($P<0.001$).

Table 1. *Mean dry matter intakes (kg) and weight gains (kg) for piglets fed on Y or BM*

	BM	Y	SED	(df)
Intake	1·13	1·21	0·025	(36)
Weight gain (observed)	1·35	1·15	0·080	(36)
Weight gain (corrected for intake)	1·44	1·06	0·067	(35)

Despite the poorer growth seen in the Y group, on examination of the gut microflora, a consistent suppression of the numbers of coliforms could be seen in the stomach and duodenum.

Table 2. *Mean log numbers (colony-forming units) of bacteria per g (wet weight) gut contents in three sites of gastrointestinal tract taken from eighteen pairs of pigs*

Site	Coliforms				Lactobacilli		
	BM	Y	SED	(df)	BM	Y	SED
Stomach	7·15	3·19	0·212	(28)	6·86	7·94	0·376
Duodenum	6·45	3·89	0·268	(28)	6·48	7·81	0·355
Colon	9·06	7·67	0·318	(28)	6·73	8·10	0·398

The yoghurt bacteria do not colonize the porcine gut and it is suggested that the observed effect on coliforms is largely due to the acid component of the yoghurt.

Yoghurt appeared to be nutritionally less adequate for growth than the base milk from which it was derived and it is not clear why this should be so. The suppression of coliforms in the porcine intestine may have some significance in areas where there is coliform-related disease.