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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Three Hundred and Fifth Meeting of the Nutrition Society was held at the Royal Society of Medicine, Wimpole Street, London W1M 9AE on Tuesday, 24 May, 1977, when the following papers were read:

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The energy values of carbohydrates: should bomb calorimeter data be modified? By R. J. JACKSON and W. B. DAVIS (Introduced by I. MACDONALD), *Beecham Products Research Department, Randalls Road, Leatherhead, Surrey*

Allen *et al.* (1966) and Brook & Noel (1969) found that glucose or starch was less fattening than sucrose to baboons fed diets calculated as isoenergetic from bomb calorimetry.

Since fat accumulation results from that fraction of consumed energy which exceeds the energy expended, a small error in energy consumption calculations would lead to a disproportionately large difference in expected fat accumulation.

Bomb calorimetry may misrepresent the available energy of carbohydrates for three reasons. (1) Bomb calorimetry results imply that all carbohydrates will be converted to carbon dioxide and water without intermediate hydrolysis. In fact, hydrolysis occurs during the digestion of certain carbohydrates and when the carbohydrate is in excess of the energy requirement the conversion to carbon dioxide and water is incomplete. Some of the excess energy is stored as fat. (2) It is more realistic to use ΔG or 'energy' values rather than ΔH or 'heat' values of the reaction; the former incorporate an entropy term which may account for steric factors. (3) The energy contribution of sucrose may be higher than that of starch because more energy is obtained from the hydrolysis of sucrose to glucose and fructose than when starch is hydrolysed to glucose.

We suggest that the energy values of carbohydrates should be related to both the weight ingested and the free energy change associated with the metabolism to an intermediate, common point for either fat accumulation or conversion to physical energy. We have chosen pyruvate and included energy changes associated with ATP/ADP conversions.

The table summarizes the free energy changes associated with the conversion of glucose, fructose, sucrose and starch to pyruvate. The weight ratio of each carbohydrate for diets providing equal metabolic energy calculated as suggested is compared with the ratio as calculated conventionally. Glucose is considered as the reference material.

	Glucose	Fructose	Starch	Sucrose
ΔG/kJ per kg	−412·5	−423·4	−512·3	−525·5
Wt ratio (free energy)	1·00	0·974	0·805	0·785
Wt ratio (bomb calorimetry)	1·00	0·997	0·890	0·944

Thus, we suggest that weight for weight sucrose will contribute more metabolic energy than glucose, fructose or starch and we would expect glucose to be the least fattening of these carbohydrates. This has been observed experimentally but not adequately explained theoretically.

The authors are indebted to I. Macdonald for constructive help.

Allen, R. J. L., Brook, M., Lester, R. E., Sim, A. K. & Warwick, M. H. (1966). *Nature, Lond.* **211**, 1104.
Brook, M. & Noel, P. (1969). *Nature, Lond.* **222**, 562.

A study of the energy balance of a woman on varying energy intakes during 14 months. By N. N. GHALI and J. V. G. A. DURNIN, *Institute of Physiology, University of Glasgow, Glasgow G12 8QQ*

Studies of energy balance on varying levels of energy intake have been made on one woman during a continuous period of 14 months. Eleven separate periods were involved, each lasting for several weeks. These periods were divided into different levels of energy intakes: (1) 'free intakes' when the subject ate to satisfaction with no restrictions, (2) 'normal intakes' when energy intake was controlled to balance energy expenditure, (3) 'restricted intakes' when there was an attempt to produce a deliberate energy deficit of about 2·94 MJ/d, and (4) 'high intakes' where an endeavour was made to have an excess intake of about 2·94 MJ/d. The arrangement for the different periods was as follows (MJ/d): (1) 'free' intake, 7·26; (2) 'low', 4·33; (3) high, 10·33; (4) 'free', 7·4; (5) 'controlled normal', 8·61; (6) low, 5·25; (7) high, 11·13; (8) 'controlled normal', 8·19; (9) high, 12·6; (10) low, 5·25; (11) 'free'. Thus, each level of energy intake was preceded and succeeded by each of the others and interactions between them could be assessed.

During the whole study measurements were made of (a) daily body-weight, (b) body composition by densitometry in triplicate at least once weekly, (c) resting energy expenditure in the fasting state two or three times weekly in triplicate, (d) energy expenditure in the fasting state while walking on the treadmill at 2·5 mph (4 km/h) two or three times weekly in duplicate, (e) energy expenditure in many normal activities such as walking, sitting, 'sitting activities', standing and 'standing activities', (f) energy expenditure after meals of 1·26 and 2·94 MJ to estimate the effect of the 'low' and the 'high' intake periods on the degree of the increase in metabolic rate caused by the meals.

Energy intakes were measured by weighing each single item of food consumed by the subject throughout the whole 14 months. Energy expenditures were assessed from a 'diary' record of all the activities of the subject together with the energy expenditures of these separate activities.

Considerable fluctuations in body-weight, body composition, and metabolic rate have occurred throughout the study which have not always coincided with theoretical expectations. These will be discussed.

Nutrient intakes in relation to income levels in Ireland. By P. K. UPTON, *Department of Veterinary Physiology and Biochemistry, University College, Dublin* and M. J. GIBNEY, *Department of Animal Nutrition and Biochemistry, The Agricultural Institute, Grange, Dunsany, Co. Meath, Ireland*

Estimates of the nutrient intakes by twelve different income groups in Ireland in 1973 were obtained from data for expenditure on 129 food items published by the Central Statistics Office (1976). In general nutrient intakes were greatest among the lower income groups and tended to decline as income increased and then to remain constant. The dietary consumption of all nutrients examined (energy, protein, calcium, iron, vitamins A, C, D, thiamine, riboflavin and nicotinic acid) were in excess of the recommended dietary intakes (Recommended Intakes of Nutrients for the United Kingdom, 1969), except vitamin D, where intake levels were considerably lower than the recommended amounts in some instances.

Table 1. *Dietary energy intakes and the contribution of protein, fat and carbohydrate to energy intake of different income groups in Ireland*

Disposable income per household (£/capita per week)	Energy intake (MJ/capita per d)	Contribution to total energy intake (%)		
		Protein	Fat	Carbohydrate
3·9	16·5	11·1	35·5	53·4
5·0	14·6	11·2	35·6	53·2
5·4	14·5	11·3	35·2	53·5
5·7	13·0	11·2	35·0	53·8
6·0	12·4	11·4	37·1	51·5
6·6	12·2	11·5	37·1	51·4
7·1	11·4	11·6	37·8	50·6
8·4	11·7	11·7	38·4	49·9
9·8	12·2	11·5	38·1	50·4
10·9	12·2	11·6	39·3	49·1
11·5	12·1	11·7	39·8	48·5
17·2	12·7	11·7	39·6	48·7

As a percentage of dietary energy, protein was constant, varying between 11 and 12% for all groups. The percentage of dietary energy obtained from fat ranged from 35–36% in the lower income groups to 39–40% at the highest income levels. The percentage energy derived from carbohydrate was inversely related to income, varying between 53·8% at the lower income level and 48·5% at the higher income level.

The variation in the proportion of energy obtained from dietary carbohydrates reflects the greater contribution of cereals, sugar and preserves to total energy in the lower compared with the higher income groups. The increasing portion of energy supplied by fat in the higher groups is related to the increasing fraction of energy obtained from meals taken outside the home as income increases.

Central Statistics Office (1976). *Household Budget Survey, 1973* **2**, 77.
Recommended Intakes of Nutrients for the United Kingdom (1969). London: HM Stationery Office.

Dietary intakes of fat and fatty acids in six social groups in Ireland.

By M. J. GIBNEY, *Department of Animal Nutrition and Biochemistry, The Agricultural Institute, Grange, Dunsany, Co. Meath* and P. K. UPTON, *Department of Veterinary Physiology and Biochemistry, University College, Dublin, Ireland*

Based on data compiled by the Central Statistics Office (1976), estimates were made of the daily per capita intakes of fat and component fatty acids in six social groups. Intakes of fat, expressed as a proportion of total dietary energy tended to be greatest among non-manual workers. The proportion of total energy derived from fat was positively correlated (r+0·998, P<0·01) with total fat intake with the exception of farmers and fishermen who tended to rely more on carbohydrate-rich foods (cereals, vegetables, sugar) for energy.

The mean daily intakes of the major fatty acids are given in Table 1. The fatty acid composition of dietary fat was similar for all social groups. Variations in the absolute intake of each fatty acid reflect variations in total fat intake.

Table 1. *Social variation in the per capita daily intake of fat and individual fatty acids*

	Professional managerial	Salaried employee	Other non-manual employee	Skilled manual employee	Semi and unskilled employee	Farmers, fishermen
Fat intake (g)	130	127	125	125	122	145
Fat energy (% total)	41·0	39·8	38·9	38·8	37·4	36·2
C14:0 (g)	4·9	5·0	5·1	5·1	5·1	6·1
C16:0 (g)	25·0	24·8	24·5	24·5	24·0	28·3
C18:0 (g)	15·3	14·8	14·0	14·0	13·6	15·8
C18:1 (g)	40·9	39·9	38·4	38·5	34·7	41·3
C18:2 (g)	9·3	8·6	7·7	7·5	7·4	8·8

Dairy fats were quantitatively the most important fat source and accounted for between 36·5 and 45·5% of total fat intake. Meat accounted for between 33·4 and 31·8% of total fat intake.

Central Statistics Office (1976). *Household Budget Survey, 1973* **2**, 19.

Reduction of serum cholesterol in type II hyperlipidaemia by guar gum.

By D. J. A. JENKINS[1], A. R. LEEDS[1], BRENDA SLAVIN[2], J. MANN[3] and E. M. JEPSON[4], *Medical Research Council Gastroenterology Unit*[1], *Departments of Chemical Pathology*[2] and *Medicine*[4], *Central Middlesex Hospital, London NW*10 and *Department of Medicine*[3], *Radcliffe Infirmary, Oxford*

Gums and mucilaginous forms of dietary fibre such as guar gum have already been shown to lower the serum cholesterol level in normal man (Jenkins *et al.* 1975) and experimental animals (Fahrenbach *et al.* 1964). Guar gum is a galactomannan storage polysaccharide obtained from the cluster bean (*Cyamopsis tetragonoloba*) and forms viscous aqueous solutions at low concentration.

In this study guar gum was administered for 2 weeks to ten patients with type IIa or b hyperlipidaemia. 5 g of guar gum were given before each of three meals daily either in a specially prepared soup or mixed with fruit juice or milk. No other deliberate change of diet was made. Three patients had been taking 12–16 g cholestyramine/d for more than 2 years and one had been taking 1000 mg clofibrate/d. These drugs were continued throughout the trial. Serum cholesterol levels which were measured at the beginning and end of the 2 week period had been stable for 6 to 18 months before the trial.

The mean serum cholesterol level at the beginning of the trial was 345 ± 15 mg/100 ml (mean $\pm$SEM) and after two weeks of guar gum was 308 ± 16 mg/100 ml, a fall of 10·6% ($P < 0{\cdot}01$). In the three patients who had been on long-term cholestyramine treatment the mean serum cholesterol level fell 18·7%, but there was no change in the patient already treated with clofibrate. No significant changes were seen in the serum triglyceride levels and body-weights varied by no more than 1 kg over the 2 week period.

Galactomannans are the principal forms of dietary fibre in leguminous seeds which are one of the richest sources of dietary fibre eaten by man. This report suggests that guar gum alone or in combination with cholestyramine merits further study as a potential hypocholesterolaemic agent. Since high concentrations of guar gum can be incorporated into palatable foods it is possible that food may be used as the vehicle for this hypocholesterolaemic agent.

H. J. Heinz Co. Ltd provided the soup. ARL is in receipt of an M.R.C. Training Fellowship.

Fahrenbach, M. J. & Riccardi, B. A. (1964). *United States Patent.* 3, 148, 114.
Jenkins, D. J. A., Leeds, A. R., Newton, C. & Cummings, J. H. (1975). *Lancet* i, 1116.

An investigation in rabbits of the 'immunological' theory of atherogenesis. By CAROL A. MUIR, T. G. TAYLOR and K. A. MUNDAY, *Department of Physiology and Biochemistry, University of Southampton*

Davies (1969) has suggested that an immunological mechanism may be involved in the complex aetiology of atherosclerosis in man, and it has been shown that, when high-fat diets are given, atherosclerosis develops more rapidly in animals injected with foreign proteins than in uninjected controls (Howard *et al.* 1971).

To investigate this theory experimentally, groups of four weanling New Zealand White rabbits were given one of four iso-nitrogenous (312 g crude protein/kg) iso-energetic (18·6 MJ/kg) diets in a 2×2 factorial experiment lasting 11 months, in which the types of fat and protein were the dietary variables. The diets contained 190 g/kg of either maize oil (MO) or coconut oil (CO) and the two protein sources, both derived from soya-beans were Promine D (PD) and Promosoy 100 (P100) (Central Soya, Chicago, Ill., USA). The former was thought to be highly antigenic, the latter non-antigenic (Smith & Sissons, 1975).

Analysis of variance showed no significant differences between treatments in food intake or live weight gain. A significant difference in antibody titres to soya-bean protein due to the nature of the dietary protein was, however, observed ($P<0·01$) and there was a significant interaction ($P<0·05$), reflecting the large difference in mean titres, between the two groups of rabbits given MO compared with the small difference between the CO-fed groups. (MO–PD 23·4: MO–P100 6·4: CO–PD 12·6: CO–P100 11·8 arbitrary units.) Differences due to fat were non-significant.

The aortas were stained with Sudan Black and given a score of 1–5 according to the severity of the atherosclerosis and the three animals with the highest antibody titres (mean 26·5 units) had scores of 5, 4 and 5 respectively; two of these animals were in the MO–PD and the other was on the CO–P100 group. The mean score of the remaining thirteen animals was 2·85 and their mean antibody titre 10·5 units. Many of the aortas and renal arteries were highly calcified.

The MO–P100 treatment gave the lowest mean values for antibody titres, aorta scores and for the calcium content of the aortas and renal arteries. Other treatment effects were less clear-cut but the results of this preliminary experiment are consistent with the view that the formation of antibodies to dietary protein may indeed constitute a risk-factor in the development of atherosclerosis in rabbits.

Davies, D. F. (1969). *J. Atheroscler. Res.* 10, 253.
Howard, A. N., Patelski, J., Bowyer, D. E. & Gresham, G. A. (1971). *Atherosclerosis* 14, 17.
Smith, R. H. & Sissons, J. W. (1975). *Br. J. Nutr.* 33, 329.

A survey of the attitudes of members of the Nutrition Society to current hypotheses relating diet and health. By C. L. BROWN, A. M. BROWN and D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

A simple questionnaire was submitted to all members of the Nutrition Society living in the United Kingdom and Eire (941) to find out whether they agreed with statements based on various hypotheses relating diet and health, and whether they modified their diets accordingly. The questions were concerned with the role of diet in the prevention and treatment of coronary heart disease, hypertension, obesity, dental caries, 'diseases of Western civilization', and the common cold. The answers invited were 'yes', 'no' or 'undecided', but members were asked to make brief comments on questions they found difficult to answer, or inapplicable to their circumstances. 67% of the membership responded.

A high consumption of saturated fat was thought to be the major dietary factor in the aetiology of coronary heart disease; 55% accepted this hypothesis, compared with 32% for dietary cholesterol, and 23% for sucrose. The majority (70%) of those answering 'yes' to the hypotheses concerning fat and sucrose restricted their own consumption of those nutrients. Modification of the diet was more common in men, and increased with age.

The alleged relationship between a high salt intake and hypertension was accepted by 30%, and of those, approximately half restricted their salt intake.

A high proportion of the respondents (76%) agreed with the view that a lack of fibre in the diet is responsible for many of the 'diseases of Western civilization', but, in this instance, men were less convinced than women.

By far the greatest positive response was to questions relating diet and dental health. Approximately 90% agreed that unrefined carbohydrates promote dental decay, and that fluoride has a protective action. Most of those with children who supported the latter proposition used fluoride prophylactically (80%) although only 39% of all who responded knew the fluoride content of their drinking water.

More women than men admitted to being overweight (25% *vs.* 16%) but a higher proportion of overweight men had recently attempted to lose weight (89% *vs.* 77%). The rate of failure was similar for both sexes (approximately 40%).

There was modest support for the claim that a generous intake of vitamin C helps to prevent the common cold; 22% answered 'yes', and of these, 68% supplemented their diets. Multivitamin tablets were not widely used (8% of the total).

In general, the number rejecting the hypotheses was always much smaller than the number who were 'undecided'.

The influence of breakfast habits on vitamin status in the elderly. By D. I. THURNHAM, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

Breakfast cereals fortified with thiamin, riboflavin and niacin are commonly eaten in the United Kingdom and an investigation was done to determine whether breakfast habits had any influence on vitamin status. Blood samples from elderly subjects living in their own homes were analysed by this laboratory as part of a large nutritional survey organized by the Department of Health and Social Security in 1974. Information on the breakfast eaten the same morning as the venipuncture was also collected, enabling the following groups to be identified: breakfast but no vitamin-fortified cereals (B), the cereal-eating group (VBC) and those taking no breakfast (NB). Subjects taking vitamin tablets were excluded from the appropriate analyses.

Measurements of thiamin, riboflavin and pyridoxine status were done using the red cell enzyme stimulation tests where biochemical deficiency was indicated by activation coefficients $\geqslant 1{\cdot}25$, $1{\cdot}30$ and $2{\cdot}00$ respectively. Plasma ascorbic acid was measured using the dinitrophenylhydrazine method and results $\leqslant 0{\cdot}2$ mg% ($0{\cdot}0114$ mM) were taken as biochemical deficiency. Plasma glucose was measured using potassium ferricyanide reagent on blood collected in fluoride tubes (Technicon method N-16).

Numbers of subjects biochemically deficient in the vitamins shown

	Breakfast no vitamin-fortified cereals (B)		Breakfast plus cereals (VBC)		No breakfast (NB)	
	no.	%*	no.	%	no.	%
Thiamin	98	13	6	7	4	13
Riboflavin	198	27***	8	9	7	23**
Ascorbic acid	264	34	27	31	7	23
Pyridoxine	318	43	38	44	12	40

*Biochemically deficient subjects expressed as a percentage of the total within each group. Significant difference from the VBC group **$P<0{\cdot}05$, ***$P<0{\cdot}001$.

The table shows that there were significantly fewer subjects who were biochemically deficient in riboflavin in the VBC group than in either of the other groups. With respect to thiamin, the difference between the VBC group and the B group did not quite reach $P=0{\cdot}1$ and no differences were found between the groups for pyridoxine and vitamin C status.

No other differences between the VBC group and the rest of the sample were found in either the distribution of the subjects by sex or age or other biochemical indices with the exception of plasma glucose ($P<0{\cdot}001$). The mean and SD of the 91 VBC-subjects was $5{\cdot}39\pm1{\cdot}01$ mM while for the remaining 825 it was $5{\cdot}85\pm1{\cdot}97$ mM. The significance of these results will be discussed.

The work was supported by the DHSS.

A comparison of anaemia and storage iron deficiency in working women.

By E. FAIRHURST, T. L. C. DALE and B. D. RIDGE, *Beecham Products Research Department, Randalls Road, Leatherhead, Surrey*

As a preliminary to a clinical trial, the iron status of a group of working women was determined. Venous blood samples were taken from 125 fasted female volunteers (age range 16 to 50 years) at a factory in St. Helen's, Lancashire.

Haemoglobin concentration was determined by the method of Van Kampen & Zijlstra (1965). Plasma ferritin was determined by the radioimmunoassay of Addison *et al.* (1972). Plasma ferritin is a reliable indicator of storage Fe, a concentration below 10 μg/l strongly indicating Fe deficiency (Jacobs *et al.* 1972). As additional evidence of storage Fe, percentage saturation of transferrin was determined using the combined methods of Caraway (1963) and Zak & Epstein (1965). Hence we could compare the haemoglobin status of each subject with an accurate measure of her Fe store.

Eleven subjects (9%) had haemoglobin concentrations below 12 g/100 ml blood and were therefore anaemic according to the World Health Organization definition. Nineteen (15%), were Fe deficient as judged by their plasma ferritin concentration. This judgement was strongly supported by their values of percentage saturation of transferrin. These proportions were of the same order as estimates by earlier workers using haemoglobin or transferrin saturation as indicators, but we obtained haemoglobin and plasma ferritin measures on the same women.

Of the eleven anaemic women, only six were classed as Fe deficient by their plasma ferritin concentrations and the remaining thirteen Fe-deficient women did not display anaemia.

Extrapolation from our findings leads to the conclusion that over 1·6 million of United Kingdom women of child bearing age probably are suffering from storage Fe deficiency but only about one-third (0·53 million) would reveal this deficiency as anaemia.

The authors are grateful to Professor Allan Jacobs and staff at the Haematology Department, Welsh National School of Medicine for making the plasma ferritin determinations and to Mrs Jennifer Edwards for her technical assistance.

Addison, G. M., Beamish, M. R., Hales, C. N., Hodgkins, M., Jacobs, A. & Llewellin, P. (1972). *J. clin. Path.* **25**, 326.
Caraway, W. T. (1963). *Clin. Chem.* **9**, 188.
Jacobs, A., Miller, F., Worwood, M., Beamish, M. R. & Wardrop, C. A. (1972). *Br. med. J.* **4**, 206.
Van Kampen, E. J. & Zijlstra, W. G. (1965). *Adv. clin. Chem.* **8**, 141.
Zak, B. & Epstein, E. (1965). *Clin. Chem.* **11**, 641.

New thoughts on catch-up growth. By J. L. SMART, E. A. BYRNE and J. DOBBING, *Department of Child Health, University of Manchester, The Medical School, Oxford Road, Manchester M13 9PT*

Recent findings from our laboratory on the growth of mice after a period of growth restriction during the suckling period are at variance with those which we and many others have reported for rats. This discrepancy prompted detailed scrutiny of the literature on recovery growth of different species after periods of growth restriction. A rule is now proposed, on the basis of already published evidence, for predicting the trajectory of growth after a period of nutritional deprivation.

Catch-up or convergent growth is defined here as a rate of growth in previously undernourished (PU) animals which exceeds that of well-nourished controls (C) of the same age.

Suckling mice were undernourished by feeding their mothers a daily ration of a good quality diet (Porton Mouse Diet) which was about half the amount taken by control mothers at the same stage of lactation. After weaning at 25 d all mice were fed *ad lib.* The body-weights of male mice were as follows: weaning, C 19·7±2·0 g, PU 10·0±1·0 g ($P<0·001$); 91 d, C 50·2±7·3 g, PU 43·4±3·3 g ($P<0·001$). The difference in absolute weight between the C and PU mice thus decreased from 9·7 to 6·8 g between weaning and 91 d. That is, the PU mice showed partial catch-up growth. This is in marked contrast to the continued divergent growth of male rats which have been growth-retarded during the suckling period (e.g. Widdowson & McCance, 1960).

A review of the somewhat sparse literature on growth restriction in mice reveals that the typical response to undernutrition during the suckling period is partial catch-up growth, as reported above. Strangely the discrepancy with rats has never received comment. In the mouse divergent growth is apparently a more typical sequel to prenatal growth restriction (Bush & Leathwood, 1975). It is proposed that the difference between the two species is a function of the timing of the period of growth restriction in relation to that of their bodily growth spurts (in terms of absolute increments in body-weight). That of the mouse is well under way before weaning, such that mice wean at nearly half their adult body-weight, whereas that of rats occurs almost wholly post-weaning. Hence, growth restriction during the suckling period covers a significant proportion of the mouse's bodily growth spurt, but largely precedes that of the rat.

It is proposed, further, as a general rule that growth restriction before the bodily growth spurt will be followed by divergent growth, whereas growth restriction during or after the growth spurt will result in some degree of convergent (catch-up) growth. This empirical rule appears to hold true for all species for which there is appropriate published evidence: rat, mouse, pig and man.

Bush, M. & Leathwood, P. D. (1975). *Br. J. Nutr.* 33, 373.
Widdowson, E. M. & McCance, R. A. (1960). *Proc. R. Soc. B.* 152, 188.

The effects of supplementary tube-feeding used in an attempt to increase the food intake of rats fed on a low-protein diet. By K. MADI and C. R. C. HEARD, *Clinical Nutrition and Metabolism Unit, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

Young rats offered a low-protein (LP) diet *ad lib.* (NDp:E 0·04), showed biochemical changes reminiscent of kwashiorkor. Their food intake fell to about half that of animals fed on a high-protein (HP) diet *ad lib.* (NDp:E 0·10). Rats which were pair-fed the HP diet in amounts equal to the intake of LP diet (HP–PF) showed different biochemical changes, which resembled those characteristic of fasting (Heard *et al.* 1977). The present experiments describe attempts to exacerbate the changes in LP rats by increasing food intake by supplying a maize starch–dextrinized starch solution (0·8–0·9 g/ml) by tube (Madi *et al.* 1970).

Young male rats (±70 g) were fed on the HP, LP and HP–PF diets as before. Two further groups of rats (HP–FF and LP–FF) were offered the HP or LP diets respectively in amounts equal to the average LP *ad lib.* intake and received supplements by tube equal to 20% of that quantity. A sixth group (LP–F) was offered a smaller quantity (20% less) of the LP diet (modified to keep the total intake of non-carbohydrate nutrients unchanged) and the missing energy was given by tube. Food intake was measured over a 5 week period and blood and liver analysed after a 16 h fast.

Mean food intake, final body-weight and changes in blood and livers of rats fed for 5 weeks on diets of different protein-energy values

(8 rats per group)

Diet (see text)	HP		HP–FF		HP–PF		LP		LP–F	LP–FF
Food intake (g/5 wks)	502	‡	371	‡	287		309	•••	280	319
Body-weight (g)	219	‡	139	‡	103	‡	93	•••	78	81
Blood glucose (mM/l)	5·0		5·5		5·8		6·1		6·7••	6·7•
Plasma insulin (mU/l)	15·7		15·1		11·9		9·0		11·0	11·9
Plasma protein (g/l)	70·4		69·5		66·9	‡	52·7•••		58·4	54·7
Liver fat (% dry wt)	12·8		12·2	‡	9·5•••	‡	26·1•••		20·8	25·8
Liver GPT† (units/g)	8·2		13·2	‡	38·6•••	‡	2·7••		3·2	2·3

†GPT=alanine aminotransferase.
Significance of difference between columns, ‡$P<0·01$.
Significance of difference from HP columns, •$P<0·05$, ••$P<0·01$, •••$P<0·001$.

The HP–FF rats readily accepted powdered diet and supplement and the latter was sufficient to correct abnormalities found in HP–PF rats. Tube feeding failed to increase the food intake of LP rats because they decreased their intake of powdered diet, but the carbohydrate solution led to higher insulin and glucose values in blood (Platt *et al.* 1962).

Heard, C. R. C., Frangi, S. M., Wright, P. M. & McCartney, P. R. (1977). *Br. J. Nutr.* **37**, 1.
Madi, K., Jervis, H. R., Anderson, P. R. & Zimmerman, M. R. (1970). *Arch. Path.* **89**, 38.
Platt, B. S., Halder, K. & Doell, B. H. (1962). *Proc. Nutr. Soc.* **21**, vi.

Dietary zinc deficiency and excess on carbonic anhydrase activity in reproductive organs of rats. By W. H. PARRY and S. R. R. RAO, *Department of Science, Bristol Polytechnic, Coldharbour Lane, Bristol*

Dietary zinc deficiency has been reported to cause testicular atrophy (Todd *et al.* 1934) and to arrest spermatogenesis (Millar *et al.* 1960) in rats. Although Prasad *et al.* 1967 have reported the effect of dietary Zn deficiency on some enzyme systems there has been little information on the effect of such diets on the activity of carbonic anhydrase in testis, prostate, uterus and ovary. We have investigated the effect of giving both a zinc deficient and zinc excess diet on the carbonic anhydrase activity in these tissues. In one experiment these diets were given for 21 d whilst in another experiment the Zn-deficient diet was given for 56 d.

Thirty-six Wistar rats of one sex were allocated to the three groups containing twelve rats; thirty-six Wistar rats of the opposite sex were allocated similarly. Pair-feeding was practised. The Zn-deficient diet contained 1·0 mg Zn/kg diet, whilst Zn- excess diet contained 1200 mg Zn/kg diet. Four rats from each group of twelve were killed each week for carbonic anhydrase activity measurements. Carbonic anhydrase activity was measured using the colorimetric method of Wilbur & Anderson (1948) in Enzyme Units (EU/g wet tissue).

The results showed that testis of rats fed the Zn-deficient diets for 21 d had significantly increased ($P<0·01$) carbonic anhydrase activity, mean 59·41 EU, compared with mean 43·65 EU of the pair-fed controls. No significant differences were obtained in enzyme activity of testis during the period of feeding rats the Zn-excess diet for 21 d. Giving the Zn-deficient diet for 56 d had no effect on the carbonic anhydrase activity of testis.

Carbonic anhydrase activity of uterus tissue in rats fed Zn-excess diets for 21 d was lowered to zero and was therefore significantly lower ($P<0·001$) than the pair-fed controls with a mean of 20·43 EU. No significant differences were noted in enzyme activity of the uterus during the period of feeding the Zn-deficient diet.

The carbonic anhydrase activity in prostate and ovary showed no change in activity over 21 d in either Zn-deficient or Zn-excess rats.

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Changes in water intake, urine production and salt metabolism in lead-poisoned sheep. By J. QUARTERMAN, J. N. MORRISON and E. MORRISON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Changes in kidney function and ultrastructure have been described in human subjects exposed to lead (Cramer *et al.* 1974). In the sheep pathological changes of the kidneys and oedema in Pb poisoning have been described (Quarterman *et al.* 1977), but in this work we describe some changes which occurred relatively early after the exposure to Pb.

Eight lambs, initially weighing about 38 kg, were given a diet, based on oat husks, which was low in calcium, phosphorus and sulphur (Quarterman *et al.* 1977). Four lambs received the diet supplemented with 200 mg Pb/kg.

Within 2 weeks the lambs receiving the Pb-supplemented diet were producing 40% less urine than those receiving no Pb. Over the first 3 months, that is before the food consumption of the supplemented lambs was significantly reduced, the unsupplemented and Pb-supplemented lambs had, respectively, mean daily urine volumes of 800 ± 45 and 460 ± 35 ml, mean daily water consumption of 1820 ± 85 and 1300 ± 80 ml, mean urine osmolarities of 1580 ± 60 and 1865 ± 45 mM, mean osmolar clearance $\left(\frac{V\times U}{P}\right)$ of 3840 and 2550 ml/d (calculated from means), mean extracellular (thiosulphate) spaces of $18{\cdot}2\pm0{\cdot}4$ and $22{\cdot}5\pm1{\cdot}6$% body-weight and plasma volumes of $3{\cdot}1\pm0{\cdot}1$ and $3{\cdot}6\pm0{\cdot}2$% body-weight. Food consumption and the concentrations of urea and creatinine in plasma and of creatinine in the urine were little affected by Pb supplementation. Thus while the concentrations of urea, sodium and potassium were increased in their urine, the Pb-supplemented lambs excreted less of these than the unsupplemented lambs. The evidence that the lambs given Pb were retaining urea and salts is strengthened by the increased water spaces observed in these animals.

The cause of the salt and water retention observed in these Pb-supplemented sheep is not clear. They were producing more concentrated urine than the controls receiving no Pb, yet failed to drink more water to enable them to excrete the surplus salt and urea. It is possible that the increased body water may be a consequence of altered heart function rather than altered kidney function.

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The role of phospholipids and bile in lead absorption. By J. QUARTERMAN, J. N. MORRISON and W. R. HUMPHRIES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Large increases in the uptake of lead occur when the dietary fat content is raised from about 50 to over 200 g/kg (Weyrauch & Necke, 1933; Barltrop & Khoo, 1975). We have examined the effect of dietary lipids and other additives to a semi-synthetic diet (Williams & Mills, 1970) upon ^{203}Pb uptake by rats. After 2 d exposure to test diets, offered *ad lib.* about 5 μCi ^{203}Pb was given to each rat in a small quantity of test diet. They were then given the same diet, without Pb, to appetite for 2 d when the activity remaining in tissues and the gut-free carcase of each rat was measured. All dietary treatments were compared with a control diet containing 50 g arachis oil/kg.

Our preliminary experiments showed that the stimulation of Pb uptake varied with the type of fat and probably with different batches of the same type of fat, suggesting that the composition may be important as well as the dietary content. Pure samples of a saturated (trilaurin) and an unsaturated (triolein) fat added at 150 g/kg control diet, stimulated Pb uptake to a similar degree. Pb uptake was also increased by additions of lecithin, bile salts and choline (Table 1).

Table 1. *Effect of supplementary lipids, bile salts or choline or of bile duct cannulation on the retention of oral ^{203}Pb*†

Dietary supplement (g/kg control diet)	Expt	Radioactivity (% control group mean and SE) Gut-free carcase	Kidney	Liver	Blood
Nil (Control)		100	100	100	100
Trilaurin (150 g)	a	260±34**	313±39**	365±62**	213±23**
Triolein (150 g)	a	302±36**	344±33**	516±54**	300±45**
Lecithin (5 g)	b	219±23**	189±22*	179±26*	182±19**
Mixed bile salts (5 g)	c	184±16*	200±32*	129±31	157±39
Choline (10 g)	c	129±11	153±16*	133±13	128± 7*
Bile exteriorized	d	9± 3**	5± 3**	21±10**	10± 3**

†The findings are taken from four experiments. Each figure is related to that for a control group given 50 g arachis oil/kg diet, the mean tissue and whole body activities of which are taken to be 100%. Significances ($^{*}P<0.05$, $^{**}P<0.01$) are given of differences from the control group in the original experiments.

The stimulating effect of crude dietary fat on lead absorption may thus be due partly to the lecithin or other phospholipids it contains and partly to the stimulation of bile flow which will add phospholipid and bile salts to the lumen contents. It has been shown that Pb secreted in the bile is more readily absorbed than inorganic Pb given by mouth (Cikrt & Trichý, 1975) and we have shown that in rats with the bile duct cannulated and exteriorized there was no significant absorption of an oral dose of ^{203}Pb (Table 1).

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The effect of thiomolybdate on copper metabolism in the sheep: a cautionary note. By T. T. EL-GALLAD, I. BREMNER and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Dick *et al.* (1975) suggested that the effect of molybdenum on copper metabolism in ruminants was mediated in part through the formation of thiomolybdates (TM) which mobilized tissue Cu in an unusual form, characterized by its insolubility in dilute trichloroacetic acid (TCA). However, it is not certain that this criterion gives a valid measure of the distribution of Cu in plasma.

The effect of TM in vivo on plasma Cu was determined in three adult sheep given 0·2 mg Mo as TM/kg intravenously. Heparinized plasma was collected from 15 min to 7 d thereafter. Total Cu concentrations were unchanged up to 30 min but increased by 50% over the period 1 to 24 h post-injection, whereupon they declined steadily for the next 5 d. All the plasma Cu was insoluble in TCA at 15 and 30 min, when TM was still detectable in the plasma. Concentrations of TCA-insoluble Cu were 0·91 μg/ml at 1 h and decreased from then until zero values were restored at 144 h. This change was associated with a gradual decline in plasma Mo concentrations. Despite these dramatic changes in the concentrations of TCA-insoluble Cu, ferroxidase (*EC* 1.16.3.1) activities were decreased by only 10% at 15 and 30 min after TM administration. As ferroxidase accounts for over 90% of the plasma Cu and as its activity is dependent on its Cu content, it appears that Cu-binding in the plasma was actually unchanged immediately after TM treatment. This was confirmed by fractionation of the plasma collected after 30 min on Sephadex G100. Over 90% of the Cu, but no Mo, was associated with the ferroxidase-containing fraction, which now contained no TCA-insoluble Cu. These results confirm therefore that TM administration can increase plasma Cu concentrations but show that the distribution of Cu cannot be related to the insolubility of Cu in TCA.

Table 1. *Change in plasma Cu and Mo concentrations after injection of thiomolybdate*

	Concentration in plasma (μg/ml)							
Time (h)	0	0·5	2	6	24	48	96	144
Total Cu	1·05	1·08	1·36	1·36	1·56	1·41	1·35	1·16
TCA-insoluble Cu	—	1·05	0·81	0·59	0·67	0·37	0·28	0·07
Total Mo	—	4·00	2·01	1·70	1·10	0·54	0·37	0·10

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Influence of the dietary content of molybdenum and sulphur upon hepatic retention of copper in young cattle. By C. F. MILLS, A. C. DALGARNO, I. BREMNER and T. T. EL-GALLAD, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The adverse effect of dietary molybdenum and sulphur upon copper utilization by growing cattle is being assessed.

Eighteen Friesian calves were offered a low-Cu semi-synthetic diet for 64 d to reduce the initial variability of liver Cu reserves. Mean liver Cu concentration was then 89±10·6 mg/kg dry matter (DM) and total liver Cu was estimated to be 67·4±7·8 mg. Animals were randomized into 4 groups to receive the same diet, but now with 11·9 mg Cu/kg DM, for 4 months. Ammonium molybdate and, or, sodium sulphate was added to achieve concentrations of Mo (mg/kg) and S (g/kg) in diets for the following groups: low Mo-low S (LMLS), <0·05 and 1·8; high Mo-low S (HMLS), 5·0 and 1·8; low Mo-high S, <0·05 and 3·3; high Mo-high S, 5·0 and 3·3.

Increasing the S content of the low Mo diet from 1·8 to 3·3 g S/kg had no effect on liver Cu retention. A fall in Cu retention was expected from previous studies with sheep. Whether this is a species difference or arises because of the very low Mo content of our basal diet is not known. The magnitude of the effect of treatment HMHS in depressing liver Cu was substantially greater than anticipated and there was a net loss of liver Cu despite the high dietary content of Cu.

Increases in dietary S content significantly increased rumenal sulphide. Concentrations of rumenal sulphide were also higher when high Mo diets were offered but the difference was not statistically significant. Increases in the dietary content of Mo or S decreased the content of Cu in the soluble fraction of rumen contents.

Plasma Cu was reduced in the HMHS animals but the proportion of Cu soluble in 5% w/v trichloroacetic acid was unchanged.

Table 1. *Dietary molybdenum and sulphur; effect on liver copper retention and soluble copper in rumen of cattle*

Treatment (no. of animals)	LMLS (4)	HMLS (4)	LMHS (4)	HMHS (6)
Liver Cu (mg) gain or loss in 4 months	+110±26·8	+38±24·1	+114±16·8	−51±18·7
Soluble Cu in rumen liquor (mg/kg DM)	25·0± 3·4	8·0± 2·4	10·3± 2·1	6·6± 0·9

Salivary and pancreatic lipases of the preruminant calf. By JOYCE TOOTHILL and S. Y. THOMPSON, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The lipolysis of milk triglycerides by calf saliva was reported by Wise *et al.* (1940). Starch-gel electrophoresis of calf saliva gave several active components that were considered to be of high molecular weight (Ramsey, 1962).

For our studies, saliva was collected from two calves with oesophageal fistulas, whilst the animals drank water from a teat-bucket. Pancreatic juice was obtained from two calves by cannulation of the pancreatic duct.

Lipolytic activity was determined by the pH-stat method using, as substrates, triglyceride emulsions, that, unless otherwise stated, contained gum acacia and taurocholate.

The maximum rate of lipolysis of emulsions of tributyrin and of medium-chain triglycerides (MCT), by reconstituted freeze-dried saliva occurred between pH 6·0 and 6·5, that is, at about two pH units below the corresponding values for pancreatic juice.

The lipolytic activity of freshly collected saliva was compared using an aqueous emulsion of tributyrin, with or without taurocholate, and an MCT emulsion. The highest rate of lipolysis occurred with the tributyrin–taurocholate substrate. Lipolytic activity decreased as the volume of water consumed by the animal increased.

Five different freeze-dried saliva samples were reconstituted and chromatographed on Sephadex G100. Using tributyrin or MCT emulsions, two peaks of activity were found, the first appearing at the void volume. Chromatography of the slower moving lipase with standard proteins gave a molecular weight range of 46 000–52 000.

Chromatography of three reconstituted, freeze-dried pancreatic juice samples on Sephadex G100, and assay of the fractions using triolein gave one peak of lipase activity which was well separated from the colipase peak. Chromatography of the separated lipase with standard proteins gave a molecular weight range of 80 000–86 000 in contrast to a value of 48 000 reported by Julien *et al.* (1972). Our method of separation gave a value of about 45 000 for pig pancreatic lipase from a commercial preparation. Lipolysis of tributyrin by the separated calf pancreatic lipase was not inhibited by 4 mM taurodeoxycholate (Erlanson & Bergstrom, 1972) and consequently the addition of colipase was without effect, but under the same conditions, the separated pig lipase was strongly activated.

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A rat growth assay for the potency of foods as a source of niacin. By E. G. CARTER, R. F. HURRELL and K. J. CARPENTER, *Department of Applied Biology, University of Cambridge, Pembroke Street, Cambridge CB2 3DX*

The evaluation of chemical methods for the determination of 'available' niacin in foods depends on comparisons with the results of bio-assays. Tryptophan as well as niacin gives a response in animals and this is further modified by the over-all balance of amino acids. We have kept the total tryptophan content of each test diet constant at a level (0·09%) adequate for growth, but with little surplus for conversion to niacin; and the levels of the other essential amino acids are also controlled.

The basal diet, modified from Harris & Kodicek (1950), consisted of ground whole maize 400, gelatin 60, vitamin-free casein 35, maize oil 30, minerals 55, nicotinic acid-free vitamin mix 5, L-lysine hydrochloride 1·49, L-methionine 2·25, L-cystine 1, L-tryptophan 0·13, L-threonine 1·5, L-valine 1·1, L-isoleucine 1·7, and sucrose to 1000. The standard response curve was obtained by additions to this basal diet of 0, 1, 2, 3·5, 5 and 10 μg/g niacin. The test supplements were added at two levels calculated to contribute approximately 2·5 and 5 μg niacin/g respectively. These additions were balanced by removal of quantities of casein, gelatin and amino acids that kept the levels of nitrogen and of essential amino acids constant. The samples were analysed for N and for tryptophan, and literature values assumed for other amino acids.

After weaning at 21 d, the rats are depleted for 10 d on the basal diet, then randomized, and four cages of two rats each are allocated to each experimental diet which they then receive *ad lib* for 14 d. The mean response has been 3 g weight gain per rat on the basal diet alone and 47 g with the highest niacin supplement. Analysis of the response using the slope ratio method (either for weight gain or gain/food) has given the following values (μg niacin/g food dry matter):

	Wt gain		Gain/food 14 d	(Total niacin by chemical analysis (Kodicek & Wilson, 1959))
	over 10 d	over 14 d		
Autoclaved *Phaseolus vulgaris* beans	29·3±3·1	31·9±3·0	29·8±3·2	(26·2)
Freeze-dried sweet corn	45·5±6·5	41·0±5·7	38·1±7·1	(37·3)
Autoclaved and freeze-dried sweet corn	44·9±7·1	45·5±6·3	48·5±8·0	(40·6)

The assay conditions appear satisfactory and the coefficient of variation of the estimate at approximately 15% is similar to that commonly found with weight gain assays.

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