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ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Ninety-second Scientific Meeting of the Nutrition Society was held at Llandaff College of Education and Home Economics, Llantrisant Road, Llandaff, Cardiff, on Thursday, 8 April 1976, at 14.00 hours, when the following papers were read:

A preparation of field bean (*Vicia faba* L.) cotyledons as a substitute for dried skim-milk in calf feeding. By I. F. DUTHIE, *Twinoaks, Cobham, Surrey,* E. OWEN, *Department of Agriculture and Horticulture, University of Reading,* E. L. MILLER, *Department of Applied Biology, University of Cambridge,* and B. M. LAWS and M. J. OWERS, *Pauls & Whites Foods Ltd, Ipswich*

Methods of processing legume seeds, such as field beans (*Vicia faba* L.), to provide dried skim-milk (DSM) substitutes for feeding to calves and other young mammals, have been described (AP & VP Ltd, 1974). For example, a preparation was made by hulling field beans (var. Maris Minor), grinding the cotyledons to a flour, treating the flour enzymically (amylolytic) in aqueous slurry to degrade the starch, incorporating hydrogenated vegetable oil and drying using commercial-scale spray-drying equipment. The free-flowing, fat-filled, powdered product (Fabalac; AP & VP Ltd, Woking, Surrey) obtained was buff in colour, bland in odour and taste, and readily dispersible and suspensible in water. Its proximate composition (g/kg) was: Crude protein (CP) (nitrogen×6·25) 256, carbohydrate 444, fat 216, ash 43, moisture 32, fibre 7.

Three milk-substitute powders were formulated to contain (g/kg) CP 240, fat 170. The control diet (A) was based on a fat-filled DSM and dried whey; diets B and C contained 150 and 300 g Fabalac/kg respectively replacing the fat-filled DSM of diet A, with vitamin, mineral and methionine supplements to match A. The reconstituted milk-substitutes were fed in buckets to individually penned Friesian male calves twice daily at the calf units of Reading (1) and Cambridge (2) Universities and once daily at the Akenham Research Centre of Pauls & Whites Foods Ltd (3) during the period November 1974 to March 1975. Five home-bred, five bought-in and ten bought-in calves/diet were used at centres 1, 2 and 3 respectively. Mean starting body-weight was 42·4 kg, starting age was 4–5 d (centre 1) or less than 7 d (centres 2 and 3) and the experimental period per calf to weaning was 35 d. Calf starter pellets, hay and water were provided throughout.

The milk-substitutes were accepted readily and were rapidly and completely consumed. No signs of digestive upset (scouring) attributable to the treatments were observed. Results for twice-daily-fed calves at centres 1 and 2 were combined. Differences in live-weight gains between treatments were not significant ($P>0·05$). Adjustment, by covariance, of live-weight gains for differences in starter pellet intake increased the precision of the experiment but

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differences between treatments for both once- and twice-daily fed calves remained non-significant. The results, mean values for ten calves/treatment, were:

Feeding schedule ...	Once/d				Twice/d			
Diet ...	A	B	C	SEM	A	B	C	SEM
Milk powder intake (kg/35 d)	13·2	13·3	13·3	—	13·6	13·7	13·7	—
Starter pellet intake (kg/35 d)	14·3	18·7	17·0	1·61	17·7	17·8	15·7	1·81
Body-weight gain (kg/35 d)	14·6	17·4	15·6	1·13	18·6	18·5	16·5	1·25
Adjusted body-weight gain (kg/35 d)	16·1	16·1	15·4	0·62	18·2	18·0	17·4	0·62

Field beans have generally been regarded as unsuitable for feeding to pre-ruminant calves because of their high fibre and starch contents. However, the satisfactory results now obtained demonstrate that when processed relatively simply and economically as described, field beans, as a source of both protein and energy, can replace a substantial part of DSM without detriment to the physical, nutritive or safety properties of the milk-substitute for calves from the first week of age. These results indicate the feasibility of using similar preparations for other young mammals, including the human infant.

REFERENCE

AP & VP Ltd (1974). UK Patent Application no. 06843.

Evaluation of the quality of a soya-bean-protein concentrate by nitrogen balance studies with human volunteers. By W. VAN DOKKUM and R. LUYKEN, *Central Institute of Nutrition and Food Research TNO, Zeist, The Netherlands*

In the course of two nitrogen balance studies some quality aspects of a soya-bean-protein concentrate (500 g protein/kg dry matter) were investigated. FAO/WHO (1971) recommends a safe level of intake of 0·57 g protein (milk or egg protein)/kg body-weight per d for male adults. This level formed the basis of our study.

Ten healthy, adult volunteers were given a diet containing 0·6 g protein/kg body-weight per d: the test product supplied 80% of the protein intake, 50% of which was used for breakfast and lunch as a spread on protein-free bread. The cooked meal contained the remaining soya-bean-protein concentrate in a sauce served with protein-free macaroni or spaghetti.

Energy balance was maintained throughout the experiments, which consisted in the first instance of seven consecutive balance periods of 5 d each, and in the second, of ten periods of 5 d each, preceded by 21 d adaptation.

The diet was supplemented by a mineral–vitamin tablet given daily. The meals were prepared and served at the Institute. The N content of the urine, stools and foods was determined using the Kjeldahl method. Carmine and polyethylene glycol were given as faecal markers.

Fasting blood samples were taken at intervals and some anthropometric data indicating body composition were collected during the study. The results in both

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studies show an initial negative N balance. During the experiments the volunteers reached constant positive N balance values (approx. +0·40 g/d). This indicates adaptation to a relatively low protein intake.

With healthy adults it is to be expected that the real N balance is about zero, but due to the fact that skin losses etc. are not measured (generally of the order of 3–5 mg N/kg body-weight per d; FAO/WHO, 1971), we may expect a slightly positive N balance if equilibrium is reached. We can conclude from the results that the test product can support a positive N balance and that a positive quality aspect can therefore be attributed to the soya-bean-protein concentrate.

A possible relationship between the time required to reach N equilibrium and the protein intake per kg lean body mass and the use of serum urea levels as a protein quality index (Taylor, Scrimshaw & Young, 1974), was discussed.

We gratefully acknowledge the encouragement and support of Unilever Research, Vlaardingen and Duiven, The Netherlands.

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Taylor, Y. S. M., Scrimshaw, N. S. & Young, V. R. (1974). *Br. J. Nutr.* 32, 407.

Ascorbic acid saturation levels in young and old guinea-pigs. By J. E. W. DAVIES, J. PULSINELLI and R. E. HUGHES, *Department of Applied Biology, University of Wales Institute of Science and Technology, Cardiff CF1 3NU*

There is evidence of a negative correlation between age and blood ascorbic acid (AA) in man (e.g. Brook & Grimshaw, 1968) and of a similar relationship between age and tissue AA in guinea-pigs given a standard daily dose of AA per unit body-weight (Hughes & Jones, 1971).

In the present study, tissue saturation levels for AA were compared in young and old male albino guinea-pigs. Group A contained six guinea-pigs aged >150 weeks and group B contained eight guinea-pigs aged 12 weeks. All animals received a standard scorbutogenic diet and 10 g AA/l in the drinking-water, a method previously shown to produce tissue saturation in 2–3 d (Hurley, Jones & Hughes, 1972). After 10 d the animals were killed and the AA measured in selected organs: the results are given in Table 1 which also includes comparable saturation values from previous experiments with young animals.

The old animals had lower AA saturation values than the young ones. Of particular interest is the fall in the brain AA saturation level: brain AA is unusually resistant to dietary-induced changes in the whole-body AA status. This difference in AA tissue saturation would thus appear to be a reflection of a reduced capacity of 'aged' tissue to abstract or retain AA, or both, and supports a previous suggestion that the retention of AA is perhaps a function of the metabolic activity of the tissue (Williams & Hughes, 1972). In terms of human nutrition it could also

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Table 1. *Ascorbic acid saturation levels (μg/g tissue) in old (A) and young (B) guinea-pigs together with results of Hurley, Jones & Hughes (1972) (C) and Hughes, Hurley & Jones (1971) (D) on young animals*

(Mean values with their standard errors)

Group . . .	A		B		C		D	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver	175	46	316	2·9***	410	13	—	—
Adrenals	1485	204	1864	89*	185	41	1810	65
Spleen	425	276	512	3·0**	477	22	456	13
Brain	161	65	205	2·2***	202	3·1	213	4

Values significantly different from group A: *$0{\cdot}05<P<0{\cdot}1$, **$P=0{\cdot}05$, ***$P<0{\cdot}01$.

imply that the use of 'young tissue' levels as a yardstick for detecting 'old tissue' deficiencies is scientifically invalid.

J.E.W.D. was supported by a Beechams Products grant.

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The influence of dietary ascorbic acid on the concentration of mercury in guinea-pig tissues. By D. R. MURRAY and R. E. HUGHES, *Department of Applied Biology, University of Wales Institute of Science and Technology, Cardiff CF1 3NU*

In a recent examination of the relationship between mercury and ascorbic acid (AA) it was found that 'megadoses' of AA enhanced the deposition of orally-dosed inorganic Hg in the tissues of guinea-pigs (Blackstone, Hurley & Hughes, 1974). The present experiments were designed to determine: (1) whether AA influences primarily the absorption of Hg from the gastrointestinal tract or its uptake and retention by the tissues, and (2) whether a similar relationship exists between AA and organic Hg.

In Expt 1 four groups of young male guinea-pigs received the treatments indicated in Table 1. Expt 2 examined the effect of high and low dietary AA on the tissue levels of orally-dosed methylmercuric iodide.

The results of Expt 1 indicated that a high AA intake increased the tissue levels of orally-administered Hg but was without effect on injected Hg (Table 1). This would suggest that AA enhances the absorption of Hg from the gastrointestinal tract, thereby shortening the survival time of guinea-pigs exposed to oral doses of Hg. Expt 2 (Table 2) indicated that a large AA intake had a similar, but less pronounced, effect on the absorption of organic Hg.

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Table 1. *Expt 1. Tissue mercury concentrations (μg Hg^{2+}/g wet weight) following oral or intramuscular mercuric chloride in young, male, albino guinea-pigs receiving ascorbic acid (AA) in the diet or in the drinking-water*

(Mean values with their standard errors for seven guinea-pigs/group)

Hg dose (mg Hg^{2+}/kg body-wt per d) . . .	Oral, 8				Intramuscular, 0·37			
AA dose . . .	10 mg/kg Body-wt per d		10 g/l Drinking-water†		10 mg/kg Body-wt per d		10 g/l Drinking-water	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver	14·61	4·07	26·15**	8·37	10·13	3·81	2·30	0·95
Brain	1·31	0·84	1·91	0·90	1·10	0·49	0·98	0·36
Kidney	150·97	33·38	398·43**	56·05	72·01	20·56	83·15	30·45

Significance of difference from values in column 1: **$P<0·01$

†The animals in this group had a survival time of 2·9±1·1 d, and were killed when death appeared imminent; in the other groups the animals were killed after 5 d treatment.

Table 2. *Expt 2. Tissue mercury concentrations (μg Hg^{2+}/g wet weight) in guinea-pigs given methylmercuric iodide (8 mg Hg^{2+}/kg body-weight per d) and receiving different intakes of ascorbic acid (AA)*

(Mean values with their standard errors for seven guinea-pigs/group)

	Liver		Brain		Kidney		Spleen	
AA dose	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10 mg/kg Body-wt per d	36·47	16·37	8·53	4·27	72·23	28·25	26·91	7·84
10 g/l Drinking-water	60·67**	14·75	9·07	2·48	170·48**	34·15	40·67*	11·25

Significance of difference between means: *$P<0·05$, **$P<0·01$.

AA is known to enhance the absorption and mobilization of iron and to have involvements with other metals (Hughes, 1974). Whether the effect of AA on Hg absorption falls into the same category is not known. In the meantime, exposure to high environmental Hg (and possible cadmium) should perhaps be regarded as a contraindication by advocates of megavitamin C therapy.

D.R.M. received a UWIST research studentship.

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Orange-peel flavonoids and the growth and ascorbic acid status of hypovitaminotic C guinea-pigs. By H. K. WILSON, C. HASSALL, C. PRICE JONES and R. E. HUGHES, *Department of Applied Biology, University of Wales Institute of Science and Technology, Cardiff CF1 3NU*

There is evidence of a metabolic relationship between ascorbic acid (AA) and flavonoid-rich preparations (Hughes & Jones, 1971). Recent studies have indicated that in both man and guinea-pigs an extract of orange-peel can modify AA metabolism (Wilson & Hughes, unpublished results). Experiments were designed to characterize this relationship using hypovitaminotic C guinea-pigs receiving 1 mg AA/kg body-weight per d.

In the first experiment a flavonoid-rich extract of orange-peel (50 mg/kg body-weight) increased both the growth of guinea-pigs and the concentration of AA in the tissues. After 13 d the mean body-weight (with SE) of a control group had decreased from 369±15 to 339±28 g, whereas that of the 'orange-peel' group had increased from 370±13 to 403±43 g.

In a second experiment hesperidin, a common naturally-occurring flavonoid, had a similar effect on both growth rate and tissue AA. The modification in growth rate cannot, however, with certainty be attributed to the flavonoid enhancement of tissue AA alone (Table 1).

Table 1. *Effect of orange-peel extract and hesperidin (50 mg/kg body-weight) on the tissue concentrations of ascorbic acid (AA) in guinea-pigs receiving 1 g AA/kg body-wt per d*

(Mean values with their standard errors; no. of animals in parentheses)

Group	Body-wt (g)				AA concentration					
	Initial		Final		Adrenals (μg/g)		Spleen (μg/g)		Leucocytes (μg/10^8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	430 (8)	10	459 (8)	40	60·7 (8)	4·2	19·5 (8)	1·7	6·22 (8)	0·32
Orange-peel extract	417 (8)	12	516 (7)	55	95·3*** (7)	4·9	27·4** (7)	2·4	7·97*** (7)	0·48
Hesperidin	420 (8)	10	550* (8)	23	91·6*** (8)	3·4	24·7 (8)	2·6	7·45* (8)	0·46

Significance of difference from value for control group: $^{*}P<0·05$, $^{**}P<0·02$, $^{***}P<0·01$.

In a third experiment hesperidin, hesperetin and flavanone were given to three groups of guinea-pigs to examine the possible relationship between biological activity and molecular structure. Hesperetin showed the greatest activity, a finding at variance with the results of earlier in vitro studies on the antioxidant activity of flavonoids (Clemetson & Anderson, 1966).

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The role of ascorbic acid in allergic reactions. By C. W. M. WILSON, *Department of Pharmacology, Trinity College, Dublin 2, Republic of Ireland*

It is known that ascorbic acid (AA) plays a role in the prevention of allergic reactions in guinea-pigs and man (Long, 1950; Zuskin, Lewin & Bouhuys, 1973), but no evidence has been provided about the mechanism by which it produces this effect. Leucocytes normally take up AA after incubation in an AA, buffered medium (Loh & Wilson, 1970). When leucocytes from atopic individuals are incubated in the medium containing the allergen to which they are sensitive, leucocyte uptake of AA is significantly reduced by at least 20% in comparison with the uptake by non-atopic leucocytes as demonstrated in the Leucocyte Ascorbic Acid Direct Antigen Challenge Test (LAADACT) (Wilson, Loh & Watters, 1975). In thirty atopic patients who had positive skin tests and LAADACTs reacting to specific allergens, leucocyte and plasma AA levels were significantly reduced in comparison with subjects having negative LAADACTs (leucocyte level $P<0.002$; plasma level $P<0.02$). This indicates that a pathophysiological cell deficiency of AA occurs in atopic subjects. When results from the LAADACT are compared with those obtained by the radioallergosorbent test in atopic individuals, a significant correlation is found between the results of the two tests ($P<0.002$). This indicates that AA is associated with the antigen–antibody reaction.

AA at normal tissue concentrations reduces breakdown of cyclic AMP by potentiating adenylate cyclase (*EC* 4.6.1.1) through its effect on catecholamine synthesis. It also inhibits the hydrolytic activity of 3′:5′-cyclic-AMP phosphodiesterase (*EC* 3.1.4.17) (Lewin, 1974). Cyclic AMP mediates the release of pharmacological inflammatory agents including histamine and prostaglandin $F_2\alpha$. It has been shown that low tissue concentrations of AA are associated with increased prostaglandin $F_2\alpha$ release (Puglisi & Berti, 1975; Sharma, Pugh & Wilson, 1975) and that AA potentiates the effect of mepyramine in inhibiting delayed allergic reactions (Dawson, Starr & West, 1966). It is postulated that the reduced cellular concentration of AA during the antigen–antibody reaction is intimately involved in the production of the allergic response.

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The absorption and distribution of ^{59}Fe in chicks with acute *Salmonella gallinarum* infection. By R. HILL and I. M. SMITH, *The Royal Veterinary College, Boltons Park, Potters Bar, Herts.*

Anaemia occurs in chicks with acute *Salmonella gallinarum* infection (Mohammidi, Hill, Smith & Licence, 1976) and the experiment described here was carried out to determine if a change in iron absorption contributed to this effect.

Layer-type hybrid cocks were given a nutritionally adequate diet: no Fe supplement was included. The chicks were inoculated orally with *S. gallinarum* at 15 d of age. The birds were each given a single oral dose of $^{59}FeCl_3$ solution containing about 5 μCi ^{59}Fe, and killed 24 h later. Groups of birds, twelve infected and eight controls, were dosed on the following days in relation to inoculation: 0, 1, 2, 3, 4 and 5; just before each bird was killed the body temperature was recorded. After death the contents of the digestive tracts were rinsed out and discarded, and ^{59}Fe was determined in samples of liver, spleen and ash from the remainder of the body of each bird.

The total count of ^{59}Fe in liver, spleen and body ash was calculated as a percentage of intake and is referred to here as absorption. In control birds absorption remained high, about 18–20% of the dose in birds killed on each of the days 1 to 6 after inoculation, while in infected birds absorption fell fairly steadily during the 6 d, to about half that in control birds. Mean percentage absorption values with standard errors of differences between means for pairs of days were:

	Days 1+2	Days 3+4	Days 5+6
Infected	13·4	12·2	8·0
Control	18·8	20·0	18·2
SE of differences	2·8	2·2	2·0

The proportion of absorbed ^{59}Fe found in the livers of infected birds decreased as the disease developed, while the reverse trend occurred in corresponding spleens.

These changes in absorption and distribution of ^{59}Fe occurred as body temperature increased in infected birds and at a time when, in other experiments, serum Fe decreased and anaemia occurred. A decrease of Fe absorption in the early stages of the disease produced by *S. gallinarum*, and a marked progression of this trend as the disease developed, evidently played a large part in the change in Fe metabolism associated with the disease.

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The nutritional implications of changes in pre-packed mushrooms stored under optimum conditions. By P. W. GOODENOUGH and D. J. COOK, *Home Food Storage and Preservation Section, Long Ashton Research Station, University of Bristol, Bristol BS18 9AF*

It has been known for some time that as mushrooms develop from small closed sporophores ('buttons') to large open sporophores ('flats') the amount of *o*-diphenol

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oxidase (*EC* 1.10.3.1) activity per unit weight increases. This enzyme can cause a browning reaction but it is not released from the fungal hyphae until dissolution of the sporophore after spore discharge, when the whole body turns brown and black. Very recent work has shown that the storage carbohydrates and polyols (mainly mannitol) increase until maturity and then decrease during fungal decay.

The activity of *o*-diphenol oxidase/unit weight was used as a marker of physiological age in mushrooms stored at 0, 10 and 25° (all at 45–55% relative humidity in perforated plastic-film packs). The age of mushrooms could be assessed visually by a trained observer. However, the pattern of enzyme activity revealed a subtle change which is invisible. At 25° the mushrooms grew rapidly, with elongation of the stalk and opening of the cap occurring, but after 5 d they had begun to decay. The enzyme assay showed a twofold increase in activity followed by a subsequent fall as decay started. Even when held at 10° or 0° the mushrooms still showed a physiological ageing comparable to that at 25°, but the rise in activity took place more slowly. Although the physical appearance of the mushrooms changed slightly at 10° there was no discernable change at 0°. Experiments on the concentration of storage carbohydrates present indicate that the fungus continues to respire using these compounds as an energy source.

After 9 d of storage at 10° the mushroom is still edible, but presumably devoid of nearly all storage carbohydrate, and is senescent. If left overnight at 25° this age of pack would immediately rot and become useless, yet it could be bought by a consumer to whom it would appear physically palatable. At 0° the pattern was similar to that at 10°, except that enzyme activity did not reach the same concentration and took an extra 2 d to disappear. Again the physical appearance of these mushrooms did not change significantly during 14 d of storage, although physiological maturing and senescence occurred. Once again a short time at 25° would lead to immediate rotting. Furthermore the taste of the 14-d-old fungus was acceptable until compared with that of fresh produce, when it was realized that the stored fungus had developed 'off odours'.

Insulin secretion in young and adult offspring of rats given diets of varying protein and sucrose content during pregnancy and lactation.

By M. R. TURNER and J. S. BRYANT, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

Variations in the postweaning diet are known to influence endocrine and metabolic function, but there are few data on the long-term effects on the offspring of small variations in the maternal diet.

Wistar rats of about 250 g were mated and then given one of four diets, high (HP) or low (LP) in protein (200 or 110 g/kg) and high (HS) or low (LS) in sucrose (300 or 50 g/kg) which on an energy basis represents (% total energy) protein 23 or 12, sucrose 36 or 6. Offspring were all weaned onto a high-fat–high-sucrose–adequate-protein regimen intended to simulate approximately the human nutritional situation in the UK. The diet provided (% total energy) fat 40, sucrose 30, protein 15, with sucrose supplying 65% of the energy from carbohydrate.

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The litter size at birth was about nine pups/litter in all dietary groups. Birth weight tended to be reduced in LP groups but the differences were not statistically significant. At weaning, the mean body-weight of offspring of LP mothers was significantly reduced ($P<0.001$). The offspring of HS-fed mothers were significantly heavier ($P<0.05$) than their LS counterparts (mean values with their standard errors: HP-HS 55.4 ± 1.2, HP-LS 46.4 ± 2.3, LP-HS 37.5 ± 2.2, LP-LS 40.6 ± 3.7 g). Food intake was similar in all groups throughout lactation (week 1, 24; week 2, 28; week 3, 34 g/rat per d).

In adult males there were no major weight differences attributable to sucrose feeding in the mothers, but the male offspring of LP mothers were significantly lighter than their HP counterparts. In females, the offspring of HP-HS mothers were heavier than other groups.

Insulin secretion was measured in vitro. At birth, there was a significant insulin increment due to glucose (3 mg/ml) in the offspring of HP mothers, but no response at all in LP offspring. The insulin increment was greater in the offspring of sucrose-fed animals.

At weaning, the basal insulin secretion was reduced in both LP groups to half that of HP animals but it was only in the offspring of LP-HS rats that the secretory response to glucose was impaired. In adult males, as in weanlings, basal secretion was halved in the offspring of LP animals, and again it was in the LP-HS group that the secretory response to glucose was deranged, but in this instance there was an exaggerated insulin secretion. In adult females, no such differences in basal nor in glucose-stimulated secretion were observed.

We are grateful to the British Diabetic Association for a grant.

Dietary protein and energy as determinants of foetal growth in the rat.

By D. J. NAISMITH, D. P. RICHARDSON and CAROLYN D. RITCHIE, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

A poor maternal diet is recognized as a major factor contributing to low birth weight. Attempts to distinguish between the effects of a deficiency of protein and a deficiency of energy on the growth of the foetus have, however, been frustrated by the failure to take account of the relationship between protein utilization and energy intake (Zamenhof, van Marthens & Grauel, 1971; Rider & Simonson, 1973).

Dietary intervention experiments on marginally undernourished pregnant women have failed to demonstrate a beneficial effect from protein, but (substantial) energy supplements appear to raise the mean birth weight and reduce the proportion of infants weighing less than 2500 g at birth (Blackwell, Chow, Chinn, Blackwell & Hsu, 1973; Lechtig, Habicht, Yarbrough, Delgado, Guzman & Klein, 1975).

The prime role of energy was illustrated in our first experiment. Rats fed throughout pregnancy on a diet containing 150 g casein/kg but restricted to the food intake of non-pregnant litter-mate controls had pups that were lighter (−16%) and contained 8.9% less protein and 8.7% less DNA than those of

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mothers given the same diet *ad lib.* Increasing the protein concentration in the diet to provide the same protein intake as that of the mothers fed *ad lib.* had no effect on the body-weight or on the protein or DNA content of the pups.

The rat foetus has a specific requirement for glucose as its energy source, whereas the adult animal can use either glucose or fatty acids. Thus feeding a carbohydrate-free diet during pregnancy would deprive the foetus of energy, but not the mother. In the second experiment, eight litter-mate pairs of rats were mated, and one of each pair was fed on a carbohydrate-rich diet containing 90 g casein/kg, fortified with methionine. The second rat received a carbohydrate-free diet providing the same proportion of energy from protein, and the animals were pair-fed with respect to energy. The experiment was terminated after 21 d. A significant difference was found in the concentration of glucose in the maternal plasma between rats receiving carbohydrate and those deprived of carbohydrate (4·50 and 3·27 mmol/l respectively), but no difference was found in the concentration of amino acids. Foetuses of the mothers given the carbohydrate-free diet were significantly lighter (−7·3%) and contained significantly less protein (−10·4%). These experiments suggest that a deficiency of energy rather than of protein may be the major determinant of foetal growth in mild chronic undernutrition.

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Are long-chain polyunsaturated fatty acids necessary in the human diet?

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A source of long-chain polyunsaturated (LCP) fatty acids (FA) appears to be necessary for optimum health in the cat (Rivers, Sinclair & Crawford, 1975). The plasma choline phosphoglycerides (CPG) of cats fed on a diet devoid of LCP FA did not contain any of these compounds, suggesting that the cat is unable to produce LCP FA from the parent short-chain polyunsaturated (SCP) FA. The mammalian brain contains considerable amounts of LCP FA and any impairment in their supply could affect the nervous system. LCP FA are associated with the membrane lipids in the brain and alterations of physical and functional characteristics may occur with changes in LCP FA composition. Erythrocyte lipids are almost entirely associated with the membrane and can be easily studied. LCP FA are found in animal products and are absent from higher plants (Crawford & Sinclair, 1972). We have investigated man's ability to produce LCP from SCP FA by studying vegans, whose diet contains no LCP FA, in comparison with omnivores. We present here some of our preliminary findings.

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Vegan subjects were contacted through the Vegan Society and matched by age and sex with omnivore controls. Dietary information was obtained and the FA composition of the plasma CPG and erythrocyte lipids were determined. The results (mean values with their standard errors for six subjects/group) were:

	FA (mg/g total FA)					
	Total polyunsaturated	SCP	LCP	20:3ω6	20:4ω6	22:6ω3
Vegans						
Mean	514·63	341·90	172·73	23·96	108·11	14·98
SE	19·07	20·18	3·94	3·70	1·95	3·60
Omnivores						
Mean	443·73	251·95	191·78	30·11	90·88	38·65
SE	14·28	12·59	12·52	1·95	6·90	4·12
Significance of difference	$P<0.02$	$P<0.005$	NS	NS	NS	$P<0.005$

NS, not significant.

In the plasma CPG the proportions of total polyunsaturated and SCP FA were considerably greater in the vegans than in the controls but the proportion of LCP FA was similar in both groups. There was no difference in the proportions of the prostaglandin precursors γ-dihomolinolenic acid (20:3ω6) and arachidonic acid (20:4ω6) between the two groups. The proportion of docosahexaenoic acid (22:6ω3) was significantly lower in the vegans and was not replaced by docosapentaenoic acid (22:5ω6) supporting the suggestion that Δ_4-desaturase activity is low in man (Sanders & Naismith, 1976).

The results for erythrocyte lipids (mean values with their standard errors for eight subjects/group) were:

	FA (mmol/mol LCP FA)							
	20:2ω6	20:3ω6	20:4ω6	22:4ω6	22:5ω6	20:5ω3	22:5ω3	22:6ω3
Vegans								
Mean	19·99	56·68	580·06	197·30	7·92	7·06	62·81	60·40
SE	2·31	7·53	9·69	12·91	2·19	1·72	7·02	14·76
Omnivores								
Mean	9·56	44·81	514·91	99·53	21·16	35·39	100·70	177·06
SE	1·74	3·81	8·52	5·66	3·33	4·45	2·17	11·00
Significance of difference	$P<0.001$	NS	$P<0.001$	$P<0.001$	$P<0.02$	$P<0.095$	$P<0.005$	$P<0.001$

NS, not significant.

In the erythrocyte lipids the proportions of 20:2ω6, 20:4ω6 and 22:4ω6 FA were significantly greater and the proportions of 20:5ω3, 22:5ω6, 22:5ω3 and 22:6ω3 were significantly lower in the vegans than in the omnivores. The ratio, 22:5ω3 FA: 22:6ω3 FA was $1·32 \pm 0·24$ (mean ±SE) in the vegans and $0·59 \pm 0·04$ in the omnivores; this difference was significant ($P<0·02$), suggesting that Δ_4-desaturase activity is low in man. Clinical and biochemical examination of the subjects yielded

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no evidence of ill health in any of the subjects, confirming earlier work that the health of vegans differs little from that of omnivores (Ellis & Montegriffo, 1970).

We would conclude that as yet there is no evidence that a source of LCP FA is necessary in the human diet. Whether differences in the proportions of LCP FA of the linolenic series in phospholipids are of any physiological significance requires further investigation.

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The possible nature of the hypocholesterolaemic action of a mould (*Fusarium*). By D. E. Owen, K. A. Munday, T. G. Taylor and M. R. Turner, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

The inclusion of a *Fusarium* mould (Lord Rank Research Centre) in the diet of hamsters or rats has been shown to increase the excretion of bile acids and neutral sterols in the faeces, and to reduce the plasma cholesterol concentration (Owen, Munday, Taylor & Turner, 1975*a*, *b*).

A hypocholesterolaemic action attributed to a water-soluble component has also been observed when edible fungi, which are related to moulds, are included in rat diets (Kaneda & Tokuda, 1966). We have measured the hypocholesterolaemic potency of extracts prepared from the mould sequentially using diethyl ether, water, ethanol–water, 30:70 and 70:30 (v/v), and ethanol as the solvent, and also of the fibre-rich residue, when these were added to a basal casein–starch–cellulose diet in an amount equivalent to 400 g mould/kg. The basal diet and a diet with 400 g mould/kg were used as controls. Diets were given for 6 weeks to groups of six weanling golden Syrian hamsters, after which plasma and liver cholesterol were determined.

The results obtained (mean values with their standard errors) were:

Diet	Plasma cholesterol (mmol/l)		Liver cholesterol (μmol/g)	
	Mean	SE	Mean	SE
Mould	1·90*	0·03	6·3	0·6
Basal (casein–starch–cellulose)	2·40	0·19	6·5	6·2
Basal+diethyl ether extract	1·72*	0·06	5·8	0·15
Basal+aqueous extract	2·64	0·16	7·3	0·7
Basal+ethanol–water (30:70) extract	2·84	0·18	5·8	0·6
Basal+ethanol–water (70:30) extract	3·31**	0·11	7·5	0·4
Basal+ethanol extract	2·32	0·16	5·9	0·4
Basal+fibre-rich residue	2·40	0·27	6·3	0·7

Significance of difference from basal diet value: *$P<0.05$, **$P<0.01$.

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Thus the hypocholesterolaemic action of the mould is confirmed. The effect is reproduced only by the diethyl ether-soluble lipid fraction. The ethanol–water (70:30, v/v) extract had a hypercholesterolaemic effect. In contrast to observations with wheat-bran fibre, experiments in vitro have failed to demonstrate any specific binding of bile acids or cholesterol by fibre prepared from the mould (Owen, Munday, Taylor & Turner, 1975*c*), which is in accord with the lack of any hypocholesterolaemic action in vivo by the fibre-rich residue. The hypocholesterolaemic action of the mould seems therefore to be attributable to a lipid component, possibly sterol in nature, which may inhibit bile acid and cholesterol reabsorption in the small intestine.

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Measurement of liver protein turnover in genetically obese mice using DL-[2-^{3}H]glutamic acid. By B. G. MILLER, R. F. GRIMBLE and T. G. TAYLOR, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

Changes in protein turnover could influence energy balance and thus contribute to obesity (Garrow, 1974). A complete description of the turnover process requires measurement of the rates of catabolism and synthesis. The former may be derived from the rate of loss of total radioactivity from tissue protein previously labelled by a tracer amino acid, and the latter from the rate of decrease in specific activity of the tracer amino acid in protein. Errors arise from reincorporation of the labelled amino acid lost from protein, and decrease both rates. It is thus necessary to ensure rapid destruction of labelled amino acid entering the amino acid pool.

The present method involves labelling tissue protein with [^{3}H]glutamic acid and measuring the rate of loss of total and specific activity of liver protein thereafter. It is valid if glutamate is the only labelled amino acid in liver protein and conditions are such that tritiated glutamate entering the pool rapidly loses its label. Theoretically the latter condition should occur as transamination and deamination remove tritium from the C_2 position on the molecule.

Twenty 11-week-old, lean and obese (obob) mice, caged individually and given a stock diet *ad lib.*, received 100 μCi DL-[2-^{3}H]glutamic acid (4·5 Ci/mmol) (The Radiochemical Centre, Amersham, Bucks.) intraperitoneally. Groups of four animals were killed by cervical dislocation on successive days after injection, and the livers were frozen in liquid nitrogen, homogenized in trichloroacetic acid (50 g/l) and centrifuged; a portion of the supernatant fraction was counted for tritium after treatment with diethyl ether and thorough drying. More than 99% of radioactivity in the amino acid pool came from tritiated water. Nucleic acids and lipids were extracted from the precipitate (Millward, 1970), and the protein was dissolved in 0·3 M-KOH and estimated by AutoAnalyzer using a modified Biuret reaction (Technicon Instruments Corporation, 1970). Portions of protein solution

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were counted for tritium after evaporation to dryness and resolubilization in water. Total and specific (disintegrations/min per mg protein) radioactivities were calculated. Analysis of hydrolysates of reprecipitated, pooled samples of liver protein showed that 85% of the label was present in glutamic acid. Calculated half-lives for synthesis for lean and obese animals were 2·92 and 4·79 d and, for catabolism, 3·04 and 5·67 d respectively. Both synthetic and catabolic rates were significantly slower in the obese than in the lean mice ($P<0·01$).

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Lipolytic and lipogenic activities of adipose tissue during spontaneous fat depletion and repletion. By I. A. MACDONALD, NANCY J. ROTHWELL and M. J. STOCK, *Department of Physiology, Queen Elizabeth College, London W8 7AH*

Fat deposition was stimulated in a group of adult male rats by twice-daily injections of insulin, whilst in a similar group of rats fat depletion was produced by restricting food intake. After 22 d these treatments were withdrawn and on days 24–27 two animals from a control group and from each experimental group were killed; animals were not fasted prior to killing. In vitro free fatty acid (FFA) release and [U-^{14}C]glucose incorporation into lipid were then determined on epididymal fat pad samples. Samples of adjacent tissue were also taken for cell sizing by the method described by Goldrick (1967) and for lipid content by a modification of Southgate's (1971) method.

Following withdrawal of the experimental treatments, the body-weights of both fat and thin animals returned spontaneously towards control levels. The average *ad lib.* energy intakes during this period were 1300, 880 and 500 kJ/kg body-weight per d for the thin, control and fat rats respectively. The most marked difference in adipose tissue metabolism was seen in the FFA release in response to isoprenaline stimulation. The release (μmol FFA/10^6 cells) for fat animals was significantly greater than for control animals ($P<0·05$), and for thin animals was significantly lower ($P<0·005$). These in vitro differences in lipolysis are reflected in the plasma FFA concentrations found in samples taken immediately after killing the animals. The FFA concentration in thin animals was lower (515 μmol/l; $P<0·01$) than in control animals (730 μmol/l) and higher in fat animals (790 μmol/l), but not significantly so.

Insulin-stimulated lipogenesis (counts/min accumulated per 10^6 cells) was greater in thin rats compared with controls ($P<0·001$), whilst in fat animals it was high initially but had returned to control values by day 27. There was no significant difference between groups in the estimated number of adipocytes per fat

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pad, thus differences expressed per 10^6 cells are maintained when expressed per epididymal pad. However, fat pad weights were very different, the mean values being 1·33, 2·70 and 3·39 g for thin, control and fat animals respectively.

Combining data from all groups reveals a negative correlation (r −0·75; $P<0·0001$) between adipocyte diameter and the energy intake on the day preceding slaughter. If this were a causal relationship, it would be compatible with the concept of a lipostatic control of food intake. Over all, this study indicates that both energy intake and adipose tissue metabolism operate to restore body fat mass towards normal following experimentally-induced deviations.

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The role of biotin in the stress-induced death of chickens exhibiting fatty liver and kidney syndrome. By A. R. JOHNSON, R. L. HOOD, JUDITH A. PEARSON and A. C. FOGERTY (introduced by D. J. NAISMITH), *CSIRO Division of Food Research, North Ryde, New South Wales* 2113, *Australia*

When chickens are reared on a diet deficient in biotin, the disorder known as fatty liver and kidney syndrome (FLKS) may appear (Payne, Gilchrist, Pearson & Hemsley, 1974; Whitehead & Blair, 1974; Pearson & Hemsley, 1976). The syndrome is characterized by an enlarged liver, an increased content of lipid in the liver, an increased level of palmitoleic acid in the liver lipids, hypoglycaemia, and often death (Johnson, Pearson, Fogerty, Shenstone, Kozuharov, Pitt & Gipps, 1972).

It has now been established that a marginally low level of available dietary biotin, which is reflected in the concentration of biotin in the liver, is sufficient to explain the malfunctions in carbohydrate and lipid metabolism which can lead to the death of stressed chickens affected with FLKS.

The normal concentration of biotin in the liver is approximately 2·0 mg/kg, but when this drops below 0·8 mg/kg, there are differing effects on the activities of two biotin-dependent enzymes, acetyl-CoA carboxylase (*EC* 6.4.1.2) and pyruvate carboxylase (*EC* 6.4.1.1). The activity of the latter enzyme is insufficient to remove pyruvate completely via gluconeogenesis and pyruvate would accumulate unless removed by other pathways. To maintain homeostasis the liver develops hyperfunctional hepatomegaly i.e. it increases in size and increases the activities of those enzymes involved in alternative pathways for the removal of pyruvate. This is reflected in an accumulation of blood lactate, removal of acetyl-CoA through increased synthesis of fatty acids and the accumulation of palmitoleic acid. These steps are accomplished by increased activity of acetyl-CoA carboxylase, even though this enzyme is biotin-dependent, malate dehydrogenase (decarboxylating) (*EC* 1.1.1.40), which supplies reduced NADP for fatty acid synthesis and the desaturase enzyme which introduces a double bond into saturated fatty acids.

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When the biotin level in the liver is below 0·35 mg/kg and the bird is subjected to stress the physiological defence mechanisms are inadequate to maintain homeostasis and finally collapse, resulting in accumulation of triglycerides in the liver and blood; the bird is unable to maintain blood glucose levels and death occurs, often only a few hours after the imposition of the stress.

The possibility that stress-induced deaths may occur in other animal species when the diet is marginally deficient in biotin was discussed, as was also the validity of assaying the vitamin status of an animal by estimation of the activities of the relevant enzymes.

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