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ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Fifty-ninth Scientific Meeting of the Nutrition Society was held in the Atkins Building, Queen Elizabeth College, Campden Hill, London W8 7AH, on Friday, 28 September 1973, at 14.15 hours, when the following papers were read :

Effect of feeding with hydrogenated coconut oil on platelet function in rats. By LILIAN McGREGOR (introduced by D. J. NAISMITH), *Queen Elizabeth College, London W8 7AH*

Diets high in hydrogenated coconut oil (HCO) have been used as thrombogenic diets although they lack essential fatty acids (EFA) (Nordöy, Hamlin, Chandler & Newland, 1968).

The purpose of this work was to study the effects of a diet high in HCO on maximal rate of shape change ($V_{S_{max}}$, arbitrary units/min per 10^8 platelets) (Michal & Born, 1971), and on the maximal rate of aggregation ($V_{A_{max}}$, transmission units/min per 10^8 platelets) (Born, 1962) of rat platelets in vitro after adding ADP.

The first experiment lasted for 8 weeks. Thirty adult male Sprague-Dawley rats were given diets containing 400 g/kg of either HCO or maize oil. The diets were otherwise adequate in nutrients except for EFA. $V_{A_{max}}$ was significantly slower on HCO, as was $V_{S_{max}}$ (see Table 1).

Table 1. *Effect on body-weight gain, no. of platelets, $V_{S_{max}}$, $V_{A_{max}}$ and packed cell volume of diets containing hydrogenated coconut oil (HCO) and maize oil*

(Median values with ranges in parentheses)

Diet	Wt gain (g)	No. of platelets (10^8/ml)	$V_{S_{max}}$ (arbitrary units/ min per 10^8 platelets) (1 μmol ADP)	$V_{A_{max}}$ (transmission units/ min per 10^8 platelets) (1 μmol ADP)	Packed cell volume (%)
HCO	110	15·4	215	54	45·6
	(90–121)	(15·2–15·6)	(85–245)	(29–57)	(42·6–53·2)
Maize oil	158*	10·5*	350*	111*	41·3
	(155–168)	(9·5–13·2)	(260–370)	(82–114)	(40·2–48·1)

*$P<0.05$.

A second experiment was carried out in the same way but with fifty-six rats for 4 weeks. The results were similar but less striking than in the first experiment.

These findings are unexpected but similar results have recently been reported by Hornstra (1973) and Nathaniel, Nathaniel, Nordöy & Chandler (1972).

The mechanisms will be discussed and include the influence of EFA deficiency on platelet membranes.

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Effects of omitting dietary sucrose and isoenergetic substitution of starch in primary type IV hyperlipoproteinaemia. By J. I. MANN, *Regius Department of Medicine, Oxford University*, A. S. TRUSWELL, *Queen Elizabeth College, London W8 7AH*, E. B. MANNING and J. RANDLES, *Department of Medicine, Cape Town University, South Africa*

There is apparently no information on the effects of isoenergetic substitution of sucrose, at ordinary levels of consumption, by starch in patients with type IV hyperlipoproteinaemia.

Seven men, 30–44 years old, with high serum triglyceride concentrations (TG) and pre-β-lipoproteins were investigated as out-patients while they continued at work. They showed no other biochemical or clinical abnormality except some obesity. For the first period each patient ate his usual diet (BAS) which was carefully recorded, and contained (g/kg): protein 110–150, fat 330–420, total carbohydrates 390–550 and sucrose 12–21% total energy, depending on the patient. In the second periods all the sucrose was replaced isoenergetically by starch (−SUCR+STCH). The extra starch was withdrawn as well in the third periods (−SUCR), thus reducing energy intakes 12–21%. Diets were carefully supervised in the patients' homes. Exercise, alcohol etc. varied as little as possible throughout. Because of the frequent home visits required, patients were studied one at a time. Periods were 2 weeks long. Fasting blood samples and body-weights were obtained on days 12, 13 and 14 of each period. Serum TG and cholesterol were determined as in Mann & Truswell (1972). Patient 7 was not given the −SUCR diet because he was not overweight.

In patients 1–6 (see Table 1) serum TG changed little with −SUCR+STCH diet but they decreased with −SUCR diet. Mean TG were 305, 295 and 229 mg/ 100 ml; mean body-weights were 85·1, 85·2 and 82·4 kg and serum cholesterol

Table 1. *Effects of dietary regimen on body-weight and the amounts of serum tri-glyceride in patients with primary type IV hyperlipoproteinaemia*

Patient	% Overweight (using mean 'desirable' wt for medium frame)	Serum triglyceride (mg/100 ml)			Body-weight changes (kg)		
		BAS	−SUCR +STCH	−SUCR	BAS	−SUCR +STCH	−SUCR
1	39	238	266	199	84·0	+0·5	−2·7
2	32	313	318	274	97·2	+0·6	−2·6
3	25	238	196	137	79·6	0	−2·6
4	26	399	391	240	95·2	−0·6	−4·6
5	21	350	364	308	73·6	+0·2	−1·9
6	14	293	236	180	81·2	−0·5	−2·4
7	6	749	351	—	76·2	+0·1	—

concentrations 253, 252 and 247 mg/100 ml at the end of each period. Patient 7 had more hypertriglyceridaemia than the others and was the only patient in whom it was familial. His serum TG decreased by 47% with $-SUCR+STCH$ diet and on resuming his usual diet increased again to 534 mg/100 ml after 2 weeks.

These results suggest that patients with primary type IV hyperlipoproteinaemia are not a homogenous group. Some obese patients were not more sensitive to sucrose than to other carbohydrates at ordinary intake levels but in one patient serum TG were inducible by sucrose.

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The effect of dietary sucrose on plasma lipids and on the liver of the spiny mouse (*Acomys cahirinus*). By K. R. BRUCKDORFER, *Department of Biochemistry, Royal Free Hospital School of Medicine, London WC1* and NANCY ANN WORCESTER and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

A significant proportion of spiny mice become obese, and develop glycosuria, hyperglycaemia and hyperplasia of the β-cells of the pancreas when kept under normal laboratory conditions (Gonet, Stauffacher, Pictet & Renold, 1965). We have studied the effect of dietary sucrose on lipid metabolism in non-obese members of this Swiss colony.

For 16 weeks, thirteen spiny mice were fed on a diet containing 680 g sucrose/kg and another twelve were fed on a similar diet containing maize starch (Bruckdorfer, Khan & Yudkin, 1972). No difference was observed in growth rate between the two groups. There was, however, in the mice given sucrose, an increase in the weight of the liver and in its concentration of lipid, and a decrease in its concentration of protein (Table 1). There was also an increase in the concentration of phospholipid and

Table 1. *Liver weight, liver composition and plasma lipids in spiny mice fed on diets containing starch or sucrose*

Diet	Liver wt (mg/g body-wt)	Composition of liver (mg/g)			Plasma lipid (mg/100 ml)		
		Water	Lipid	Protein	Triglyceride	Cholesterol	Phospholipid
Sucrose	88·1	615	162	171	125	287	344
Starch	42·5	672	90	202	118	154	223
P	< 0·002	< 0·002	< 0·002	< 0·02	NS	< 0·002	< 0·002

NS, not significant.

cholesterol in the plasma, but no change in the concentration of triglyceride. The increase in liver weight could not be explained by the accumulation of fat alone. Although the concentrations of protein and water in the liver were reduced, the absolute amounts of those constituents increased, suggesting that hyperplasia or hypertrophy, or both had occurred, as has been observed in the liver of the rat (Bender, Damji, Khan, Khan, McGregor & Yudkin, 1972).

As our work was nearing completion, a relevant paper by Shafrir, Teitelbaum & Cohen (1972) was published. Their strain of wild spiny mice were also 'non-diabetic' on the normal laboratory diet, but with dietary sucrose exhibited impaired glucose tolerance, an increased concentration of plasma cholesterol and, in contrast to our strain, also an increased concentration of triglyceride.

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Insulin sensitivity of adipose tissue of rats fed with various carbohydrates.
By K. R. BRUCKDORFER, *Department of Biochemistry, Royal Free Hospital Medical School, London WC1* and S. S. KANG and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

The evidence that sucrose may play a part in causing diabetes mellitus comes from epidemiological studies (Cohen, Bavly & Poznanski, 1961; Yudkin, 1964) and also from experimental work in human subjects and in rats (Cohen & Teitelbaum, 1964; Cohen, Teitelbaum, Balogh & Groen, 1966; Cohen, Teitelbaum & Saliternik, 1972). It has been reported that rats given diets containing sucrose show a loss of sensitivity of their adipose tissue to the lipogenic action of insulin (Vraná & Kazdová, 1970; Vraná, Slabochová, Kazdová & Fabry, 1971). We have carried out experiments to establish whether this effect is associated with the fructose moiety of sucrose.

Groups of six male Sprague-Dawley rats were fed *ad lib.* on diets containing 680 g of either starch, glucose, sucrose or fructose per kg. After 30 d, the animals were killed, the epididymal fat pads were removed, and segments were incubated for 2 h in the presence of $[2-^{14}C]$glucose with or without bovine insulin. The lipids were extracted and the radioactivity was counted (Table 1).

Table 1. *Total radioactivity (counts/min) incorporated into lipid per g adipose tissue*

(Mean values and standard deviations for six rats)

Dietary carbohydrate	Without insulin	With insulin	P value
Starch	47 800 ± 17 000	114 800 ± 43 500	< 0·05
Glucose	78 100 ± 15 000	110 100 ± 9 700	< 0·05
Sucrose	37 000 ± 6 450	36 600 ± 8 670	NS
Fructose	30 700 ± 8 150	37 200 ± 11 300	NS

NS, not significant.

The results confirm that dietary sucrose results in a loss of sensitivity of rat adipose tissue to insulin, and show that this is related to the fructose component of the sucrose. Similar results were obtained with rats that were 'meal-fed' on diets with glucose and fructose.

Vraná & Kazdová (1970) reported that the lipolytic action of adrenaline on the adipose tissue of rats was not affected by dietary sucrose. We have found no difference in lipolysis induced by adrenaline or by corticosterone in rats fed with starch, glucose, sucrose or fructose.

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Interactions between amino acids in the stimulation of insulin secretion in rabbits. By M. R. TURNER, C. E. THOMPSON and K. A. MUNDAY, *Department of Physiology and Biochemistry, The University, Southampton SO9 5NH*

The secretion of insulin is stimulated not only by glucose, but also by some amino acids, glucagon and enteric hormones. There is evidence obtained mostly from intravenous infusion studies and in vitro work to suggest that arginine, lysine and leucine are potent stimulators of insulin secretion, but that other essential and non-essential amino acids have a much smaller, or zero, effect. There are also indications, which have not been defined clearly, of a possible synergism between amino acids in the stimulation of insulin secretion (Fajans, Floyd, Knopf, Pek, Quibrera & Conn, 1971).

We have studied some of the interactions between amino acids in rabbits by administering, by stomach tube, several amino acid mixtures, and measuring changes in plasma insulin concentrations using a double-antibody radio-immunoassay. In all experiments the total amount of amino acids in the intubation mixture was 0·79 g/kg body-weight. The results are shown in Table 1. It was found that: (1) amino acids in the absence of glucose did not stimulate insulin secretion; (2) when

Table 1.   *Effect of administration of glucose and glucose + amino acid mixtures on plasma insulin concentrations in rabbits.*

(Mean values with their standard errors for seven rabbits)

Intubation mixture	Plasma insulin increment (μU/ml) after:		
	20 min	40 min	60 min
Glucose: 2 g/kg	12·7± 3·5	27·9± 4·6	24·9±3·2
1 g/kg	15·4± 5·2	15·1± 5·6	11·9±2·7
Glucose (1 g/kg) plus:			
lys, arg	22·8± 5·9	36·1± 9·5	15·0±4·6
phe, his	1·6± 1·5	10·0± 4·1	8·5±3·3
lys, arg, phe, his	27·0± 6·7	34·3± 8·5	21·5±4·5
leu, lys, arg, phe, his	27·4± 9·3	62·3±15·4	42·3±9·0
leu*	32·8± 4·3	31·7± 5·2	28·5±5·5
val, met, try, thr	22·3±10·9	22·7± 7·4	22·9±6·2
lys, arg, val, met, try, thr	26·2± 9·2	36·2±11·2	25·9±8·1

*Amount present in mixture leu, lys, arg, phe, his.

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amino acids were present in the intubation mixture together with glucose (1 g/kg) the increase in plasma glucose concentration was sometimes less, but never more, than when glucose was ingested alone; (3) most of the amino acid mixtures used were capable of producing an insulin response at least as great as that produced by an additional molar equivalent amount of glucose; (4) leucine was a particularly potent insulinotrophic amino acid, the effect of which was at least additive to that of glucose, arginine and lysine; (5) the presence of non-insulinotrophic amino acids in the mixture apparently increased the insulin responses to arginine and lysine; (6) a mixture of phenylalanine and histidine inhibited the glucose-stimulated insulin secretion but, in the presence of arginine and lysine, apparently did not do so.

No evidence was found for true synergism between amino acids when given orally with glucose. The maximum possible insulin response to glucose plus arginine and lysine was similar to that for glucose alone (30 μU/ml) which is much less than that possible with glucose plus mixtures containing leucine ($>$80 μU/ml).

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A study of 'refractory' obesity. By DOROTHY A. DENT and SALLY R. PARSONAGE, *Slimming Advisory Services, Burwood House, Caxton Street, London SW1*

In any group of obese people it is common to find individuals who are apparently unable to lose weight on relatively low intakes of energy. Sometimes this occurs at the start of dietary treatment but more frequently after a diet has been followed for a few months. Such individuals seem to be able to maintain their body-weight on daily intakes of 4·2 MJ (1000 kcal) or less for long periods. This could be explained by either their energy intake being higher than claimed or their total energy expenditure being low enough to maintain energy balance.

To study this, twenty-five women who were on average 21·3 kg above their ideal weight were confined to a residential hall for 5 d and given a carefully controlled diet that provided 4·2 MJ/d. All the subjects had previously been unable to lose weight on low-energy diets even when supervised once a week by a doctor or dietitian. Before taking part in the study, all subjects weighed and recorded their food intakes for 7 d. Analysis of these records gave a mean daily energy intake of 5·2 MJ (1230 kcal). During the experiment, resting energy expenditure was measured after an overnight fast in nine subjects who claimed they maintained weight on particularly low intakes. In all instances the energy cost of resting metabolism was within $\pm$10% of the standards of the *Handbook of Biological Data* (Spector, 1954) for 'basal' metabolism. In addition, a psychiatrist conducted a number of in-depth interviews

with the subjects. Apart from one subject who gained 0·8 kg, all subjects lost between 1·1 and 3·2 kg (mean 2·1 kg) in 5 d. A follow-up study has been conducted to see whether these weight losses were maintained or improved after the experimental period. The weight losses, taken in conjunction with the measurements of resting energy expenditure and the reactions of the subjects to the diet, lead us to believe that so-called 'refractory' obesity is usually caused by failure to adhere to low-energy diets.

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Digestion of starch in the intestine of sheep. By P. THIVEND (introduced by E. R. ØRSKOV), *Station de Recherches sur l'Elevage des Ruminants, Centre de Recherches de Clermont-Ferrand, 63110 Beaumont, France*

Several workers have shown that in some instances, particularly with maize diets (Ørskov, Fraser & Kay, 1969; Thivend & Journet, 1970), the starch from cereals can escape rumen fermentation and be digested in the small intestine. However, nobody has produced convincing evidence that intestinal hydrolysis of starch produces glucose which will be absorbed into the portal blood.

To provide information on this aspect, two weaned lambs, weighing about 30 kg, were fitted with one catheter in the portal vein and another in the posterior vena cava. Each animal received two diets: one diet consisted of chopped hay, the other of ground maize (800 g/kg) and chopped hay (200 g/kg). The hay diet was given during two periods, before and after the maize diet. During the first period, lambs were fed *ad lib.* and in the other periods they received the same amount of dry matter (950 g daily) as they were eating in the first period. The food was given twice daily. The
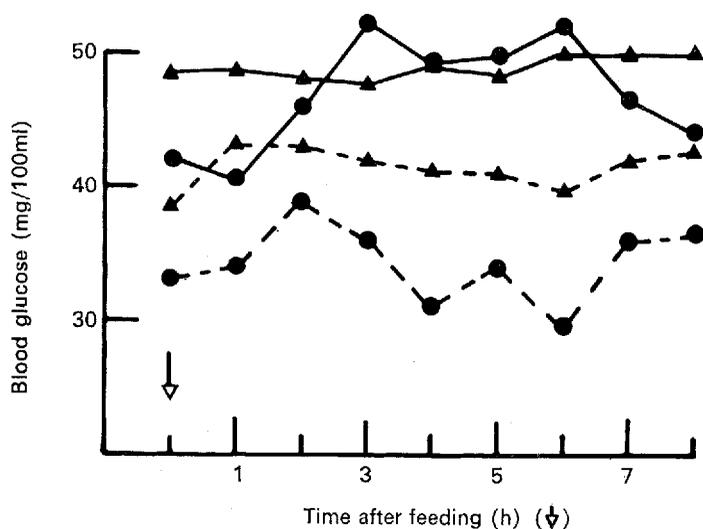

Fig. 1.   Effect on portal blood concentration of giving diets consisting of hay (— — —) or maize and hay (———) to lamb nos 1 (●) and 2 (▲).
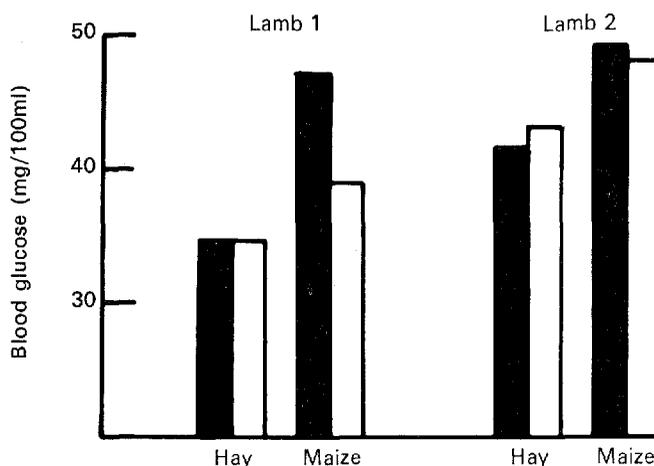
Fig. 2. Effect on mean glucose concentration in the portal vein (■) and posterior vena cava (□) of giving diets based on hay or maize to two lambs.

lambs were given each diet for 3 weeks during which time collections of blood samples were made on two–five occasions. Each collection consisted of nine samples obtained at hourly intervals after the morning feed. Blood samples were deproteinized immediately after sampling and blood glucose concentrations were determined by a modification of the glucose-oxidase method (Huggett & Nixon, 1957).

The mean results are shown in Figs. 1 and 2. Because of similarity, the results for the two hay periods were pooled. For each lamb (Fig. 1) the portal blood glucose concentration was significantly ($P<0.001$) increased (36 and 18% respectively for lamb nos 1 and 2) when maize replaced hay. There was also a substantial increase in the portal blood glucose concentration after feeding in lamb no. 1. The increase with the maize diet in the vena cava blood glucose was significant ($P<0.05$) but less (14 and 12%, Fig. 2) than the differences for portal blood. With the hay diet, the vena cava blood glucose concentration was similar to (lamb no. 1) or higher than that (lamb no. 2) in the portal blood. It is concluded that whereas the dietary differences in blood glucose concentration in the vena cava may reflect gluconeogenesis which may be greater with the maize diet (Ford, 1965), the greater differences in the portal blood glucose concentration must represent, at least partly, glucose absorption following enzyme hydrolysis of starch in the small intestine.

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Effect of changes in the glucogenic energy on yield of milk and milk fat in lactating goats. By E. R. ØRSKOV and C. FRASER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It is well known that high dietary levels of concentrates depress the yield of milk fat in dairy cows (Powell, 1939) and this effect has been ascribed to changes in the amount of gluconeogenic substances in the absorbed fermentation end-products (Rook & Balch, 1961; McClymont & Vallance, 1962; Armstrong & Blaxter, 1965).

Three lactating goats, in which the oesophageal groove reflex was maintained from birth (Ørskov, Benzie & Kay, 1970), were fed on a control diet consisting of (g/kg): 300 chopped, dried grass and 700 concentrates, or on diets in which 21% of the metabolizable energy of the concentrates was replaced by either glucose or acetic acid. The acetic acid was partly neutralized with sodium hydroxide (pH 5·0) and mixed into the feed. The glucose was given by bottle. The level of feeding adopted was constant throughout the experiment and was based on the milk yield at the beginning. The design used was a 3×3 latin square and the length of each period was 3 weeks.
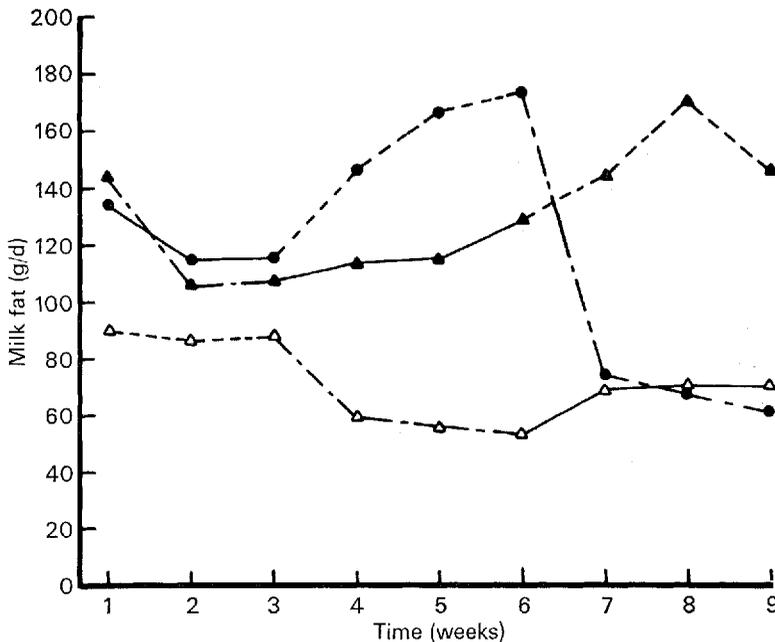

Fig. 1. Effect of acetic acid (— — —) or glucose substitutes (— · —) on yield of milk fat in comparison with that on a control diet (———) in three lactating goats.

The effect on the yield of milk fat for each goat is given in Fig. 1. Acetic acid increased and glucose decreased the yield of milk fat, the effect of glucose being almost immediate.

During the last 10 d of each period the yields of milk fat for the control, glucose and acetic acid treatments, respectively, were 105, 74 and 142 g/d (SE 16·2). The corresponding milk-fat percentages were 4·3, 3·8 and 5·4 (SE 0·26) and the milk yields were 2591, 1964 and 2609 g/d (SE 274).

The results emphasize the importance of the glucogenic energy absorbed in the partition of absorbed energy between body and milk.

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The use of inert ruthenium–phenanthroline as a digesta particulate marker in sheep. By J. C. MacRae and C. C. Evans, *Hill Farming Research Organisation, Bush Estate, Penicuik, Midlothian EH26 0PH*

[103]Ru-labelled tris(1,10-phenanthroline) ruthenium (II) chloride has been shown to be a satisfactory solid-phase marker in experiments on ruminant nutrition (Tan, Weston & Hogan, 1971; Faichney, 1972; MacRae & Ulyatt, 1972) but its general use as an intestinal marker could be somewhat limited by its radioactive properties (0.42–0.60 MeV gamma emission).

Ru can now be accurately determined by X-ray fluorescence spectrometry (Evans, unpublished results) and this has facilitated the evaluation of inert ruthenium–phenanthroline (Ru-P) as a solid-phase marker.

The complex was prepared as described for radioactive [103]Ru-P (Tan *et al.* 1971): 1 g $RuCl_3$, 0.72 g KCl, 140 ml 0.2 M-HCl and 160 ml ethanol (analar reagent) were refluxed for 20 min, the alcohol was distilled off and the solution allowed to stand overnight. The pH was adjusted by adding 20 mequiv. NaOH, and 0.7 g hypophosphite and 3 g 1,10-phenanthroline were added and the solution was refluxed for 4 h. The orange-red solution was filtered to remove specks of Ru metal and made up to 200 ml, when it contained approximately 2000 mg Ru/kg. The rate of administration of marker was controlled to give a faecal Ru concentration greater than 50 mg/kg, which allows rapid X-ray fluorescence analysis.

Faecal recoveries of Ru, measured in two sheep given continuous intraruminal 9 d infusions of the complex, were 96.6% and 101.0%. Ru was detected in faeces from day 2 to day 17, with maximum concentration occurring between days 5 and 12. In subsequent experiments, in which a pulse-dose of marker was administered intraruminally to twenty-four sheep, to measure transit time of digesta, faecal recovery of Ru was 101 ± 2.5%.

Adherence of Ru to the solid phase of the digesta was examined in the 9 d infusion experiment and also in in vitro experiments. In these, Ru-P solution was incubated with rumen liquors at 37° for consecutive 2 h periods during which the pH was adjusted to 6, 3 and 8 respectively to represent conditions pertaining in the rumen, in the abomasum–duodenom and in the ileum–caecum. When samples of rumen liquor were centrifuged at 3000 *g* for 20 min, in all instances less than 2% of the Ru was detected in the supernatant fraction. When samples of faeces excreted

on days 8–10 of the 9 d infusion were homogenized with 4 vol. water and centrifuged at 3000 **g**, the solid phase contained 58.4 ± 3.81 mg Ru/kg and the supernatant fraction contained only 1.3 ± 0.19 mg/kg.

Chromium can also be estimated by X-ray fluorescence, and infusions of Ru-P plus Cr EDTA (Goodall & Kay, 1973) have been used to study the relative transit times of the solid and liquid phases of digesta.

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The effect of dietary fibre on the response to orally administered glucose.

By D. B. JEFFFRYS (introduced by I. MACDONALD), *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

The influence of dietary fibre upon the function of the large bowel has received considerable attention recently (Harvey, Pomare & Heaton, 1973). This experiment was designed to investigate the effect of different preparations of fibre on gut function as measured by the oral glucose-tolerance test.

Six healthy subjects (four males, two females) aged 20–25 years were given, after a 12 h fast, 1 g glucose syrup/kg body-weight. Three types of 'fibre' were given: (a) unprocessed bran (Prewetts Ltd), (b) bagasse (Tate and Lyle Ltd) or (c) wood cellulose (Solkafloc, Johnson and Jorgensen). The fibre (0.2 g/kg body-weight) was soaked in water overnight and then added to the glucose solution. All the drinks were made up to 6 ml/kg body-weight with water.

Glucose drink alone was used as a control experiment, and then the 'fibre' preparations were given in a random order with a week between each experiment. Blood (0.05 ml) was taken as a free-flowing capillary sample from the ear lobe. A resting sample was obtained and then the solution was ingested as a slurry for 2 min. Further blood samples were taken at 30 min intervals for 2 h. The blood glucose was estimated by the glucose-oxidase technique of Werner, Rey & Wielinger (1970).

Table 1. *Mean values with their standard errors for increase in blood glucose concentration (mg/100 ml) above the fasting level in human subjects given different dietary fibres*

Time (min)	Control	Bran	Bagasse	Wood cellulose
30	4.6 ± 0.59	3.7 ± 0.49	6.6 ± 0.82	5.9 ± 0.43
60	4.3 ± 0.78	2.6 ± 0.35	5.9 ± 1.19	6.1 ± 0.82
90	2.8 ± 0.36	1.3 ± 0.27	2.4 ± 0.49	1.6 ± 0.56
120	1.6 ± 0.20	0.6 ± 0.32	1.3 ± 0.32	1.8 ± 0.53

The bran improved the glucose tolerance at 60, 90 and 120 min compared, within subjects, to the control ($P<0.01$) and it also reduced the area under the curve ($P<0.01$). The bagasse (90% undigestible carbohydrate) and wood cellulose (95% undigestible carbohydrate) raised the glucose concentrations at 30 and 60 min ($P<0.01$) compared to the control value.

The peripheral blood glucose values suggest that bran reduces the glucose absorption per unit time, whereas at 30 and 60 min the bagasse and wood cellulose have the reverse effect. A possible explanation might be that some of the lipid constituents of bran could slow gastric emptying, hence reducing the rate of absorption. Bagasse and wood cellulose, on the other hand, could increase intestinal motility and so the glucose would be absorbed over a greater area of the small intestine.

Thus the presence and the nature of indigestible carbohydrate in the diet can modify the metabolic response to digestible carbohydrate.

I am very grateful to the volunteers for their co-operation.

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Carbohydrate availability in human recovery from physical work exhaustion. By J. D. BROOKE, *Human Performance Laboratory, Physical Education Section, University of Salford* and L. F. GREEN, *Beecham Products, Beecham House, Great West Road, Brentford, Middlesex*

Adequate availability of carbohydrate, achieved by dietary supplement (Brooke, Davies & Green, 1972), or by physiological overadaption (Brooke & Green, 1972), results in an increased performance of physical work before exhaustion. The present paper describes the effect upon further work ability of a dietary carbohydrate supplement presented immediately after exhaustion from hours of physical work.

Three fasting racing cyclists pedalled on an Ergowheel at 55–60% maximum oxygen uptake until the non-protein respiratory quotient (RQ) indicated the available carbohydrate stores to be close to exhaustion (RQ 0.73 or incapacity for work) (Brooke & Green, 1972). The mean time to exhaustion was 147 min. In the 10 min following exhaustion the subjects consumed 250 ml glucose syrup with added salts (1486 kJ (356 kcal) energy value), a normal diet for racing cyclists, canned rice pudding with added sucrose, (1486 kJ (356 kcal) energy value) or 250 ml low-energy fluid with added salts (<20 kJ (5 kcal) energy value). A latin-square design was used. Forty minutes after exhaustion subjects recommenced

work at the same load as in the first part of the trial, and again worked to RQ 0·73 or incapacity. The amount of work done after recovery was greatest after ingestion of the glucose syrup drink and lowest on the low-energy drink; the 'normal' diet gave intermediate results.

These comparisons were replicated with three more subjects performing the same task, and a further three at 70% maximum oxygen uptake. The mean work times (nine subjects) were 80 min for glucose-syrup treatment, 58 min for 'normal' diet and 29 min for the low-energy treatment ($P<0·05$).

During the rest period between the two sets of work there was a faster increase in blood sugar concentration when the glucose syrup drink, compared with the normal diet, was taken. There was little change when the low-energy drink was taken.

Recovery from exhaustion due to reduced carbohydrate availability is more rapidly achieved with a dietary supplement of a glucose syrup drink than with a 'normal' diet or low-energy drink. In common with Christensen & Hansen (1939), we believe that the basis of this improved recovery does not lie solely in the fuel for muscle metabolism, although the effects may be seen in the blood sugar concentrations.

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Contrasting results for the reactive lysine content of heat–damaged materials. By R. F. HURRELL and K. J. CARPENTER, *Department of Applied Biology, University of Cambridge*

Finot & Mauron (1972) showed that applying different methods for 'reactive lysine' to the model Maillard compound α-N-formyl-($\in$-N-deoxyfructosyl)-lysine (FFL) gave contrasting results. Hurrell & Carpenter (1973) confirmed this with protein stored in contact with glucose. It appeared that the full extent of the heat damage in such materials was indicated by the direct fluorodinitrobenzene (FDNB) method (Carpenter 1960) but not by the FDNB difference method (Roach, Sanderson & Williams, 1967) or with trinitrobenzene sulphonic acid (Kakade & Leiner, 1969). With materials heated at high temperatures, whether or not sugar was present, the three procedures were in much better agreement. We have now applied three further analytical procedures to the same set of materials (Table 1).

Table 1. *Total and reactive lysine values for heated materials (each value expressed as a percentage of the corresponding value obtained with the unheated sample)*

Material	'Total' value after acid hydrolysis	Direct FDNB value	MIU value	Lysine after borohydride	Orange 12 binding value
FFL*	53	3	(0)†	0	ND
Ovalbumin–lactalbumin –glucose (3:2:5, by wt) (12% H_2O content):					
30 d at 37°	59	24	22	15	98
15 min at 121°	34	15	9	23	81
Bovine plasma albumin (13% H_2O content):					
27 h at c. 150°	78	14	6	81	27

ND, not determined; MIU, o-methyl isourea; FDNB, fluorodinitrobenzene.
*α-N-formyl-(ϵ-N-deoxyfructosyl)-lysine: values are expressed as percentages of the theoretical lysine content of the molecule.
†Value from Finot & Mauron (1972).

Production of homo-arginine from reaction of lysine groups with o-methyl iso-urea (MIU) followed by acid hydrolysis (Mauron & Bujard, 1963) proved a sensitive indicator with all the samples. Determination of lysine in acid hydrolysates of materials previously treated with sodium borohydride (Thomas, 1972) was a sensitive indicator for FFL itself and for both mild and severe heat with glucose, but not for protein heated alone. Though Maillard compounds may be reduced by borohydride to alkyl-lysine derivatives that are resistant to acid hydrolysis (Means & Feeney, 1968), it appears that this is not so for the linkages (hypothesized to be imides) formed when pure proteins are heated.

The adsorption of the acid azo dye Orange 12 on to proteins has been used as an indicator of reactive lysine in fish meals (A/S N. Foss Electric, 1972). With our samples, a severe reduction in adsorption is seen for the heated pure protein, but it appears that the compounds formed by reaction between protein and sugars under mild conditions still adsorb Orange 12. Presumably the lysine group still has a basic character in the latter case. We have confirmed that the material stored at 37° has only a very low nutritional value for rats and chicks. Clearly, certain procedures are suitable for measuring some types of lysine binding but not others.

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Observations on the possible nutritional significance of vitamin–binding proteins in milk. By J. E. FORD, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The folate and vitamin B_{12} in milk are strongly bound to the less important whey proteins. These 'binders' are present in excess and so milk has capacity—which varies between milks of different mammalian species—to sequester added cyanocobalamin and folic acid (see Gregory & Holdsworth, 1955; Ford, Salter & Scott, 1969; Ford, Knaggs, Salter & Scott, 1972).

A study has been made of the influence of different milks on uptake of [³H]cyanocobalamin and [³H]folic acid in selected bacteria, mostly of types that are commonly found in the intestine. None required exogenous vitamin B_{12}. Nevertheless, when free cyanocobalamin was added, in seven of nine cultures it was taken up into the cells, though there were large differences between the different bacterial species in their absorptive capacity. For example, *Escherichia coli* rapidly took up 1·84 ng cyanocobalamin/10^9 cells, as against only 0·005 ng by *Streptococcus zymogenes*. In the presence of sow's milk, which had unsaturated capacity to bind 140 ng cyanocobalamin/ml, there was little or no uptake of the added cyanocobalamin, even after incubation with the test cultures for 1 h at 37°. The avidity of sow's milk for cyanocobalamin, as judged from its retention of the vitamin against competition by bacterial cells, was considerably greater than that of a preparation of porcine intrinsic factor of similar binding capacity.

Five of the test cultures (*Bifidobacterium bifidus, Lactobacillus acidophilus* and three varieties of *Streptococcus faecalis*) required exogenous folate and rapidly took up added folic acid into the cells. Uptake after incubation for 1 h ranged from about 2·5 ng/10^9 cells in *Strep. faecalis* to 15·2 ng/10^9 cells in *B. bifidus*. When the folic acid was added with goat's colostrum, which had unsaturated capacity to bind 560 ng folic acid/ml, there was little or no uptake.

The physiological role of these vitamin-binders will be discussed. It is suggested that they act in the mammary gland to accumulate the vitamins from blood plasma into milk and in the gut to facilitate their absorption, both directly, and indirectly by preventing their uptake by intestinal micro-organisms. Thus, the binders might strongly influence the ecology of the gut microflora and the vitamin economy in the neonatal period.

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Effects of dietary cadmium and zinc on rats maintained on diets low in copper. By J. K. CAMPBELL and C. F. MILLS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The effects of high dietary concentrations of cadmium and zinc on rats maintained on diets marginally adequate in copper content have been examined.

Seventy-two weanling male Hooded Lister rats were randomly allocated to twelve groups and offered diets containing 0·16, 1·5, 6·1 or 18 mg Cd/kg and 30, 300 or 1000 mg Zn/kg in a 4×3 factorial arrangement. The basal diet was similar to that of Williams & Mills (1970) but with casein as the protein source. The Cu content was reduced to 2·6 mg/kg with the intention of meeting requirements for growth and haemoglobin production, while providing little margin of safety against the action of Cu antagonists. Rats had unrestricted access to the diets for 9 weeks and were weighed weekly.

The control group (0·16 mg Cd/kg plus 30 mg Zn/kg) grew normally, and no clinical effects were observed. Mean values with their standard errors for some of the measurements made on these animals were: plasma Cu, 0·71 ± 0·097 mg/l; liver Cu, 4·5 ± 0·16 μg/g fresh weight; cortical bone index (CBI) (Barnett & Nordin, 1960) 43·54 ± 0·64%.

Diets providing 30 mg Zn/kg and up to 18 mg Cd/kg had no effect on growth. Diets containing 1000 mg Zn/kg depressed growth rate ($P < 0.05$) and induced depigmentation of black hair (Keil & Nelson, 1931).

Plasma Cu concentrations were depressed by 40% ($P < 0.1$), 97% ($P < 0.001$) or 89% ($P < 0.001$) in animals receiving 1·5 mg Cd/kg, 6·1 mg Cd/kg or 1000 mg Zn/kg diet respectively. Diets providing 1·5 mg Cd/kg or 300 mg Zn/kg, markedly reduced plasma caeruloplasmin activity (Table 1). No change in haemoglobin concentration was observed.

Table 1. *Effects of dietary supplements of cadmium and zinc upon plasma caeruloplasmin activity (U/l) in rats maintained for 9 weeks on diets providing 2·6 mg Cu/kg dry matter*

	Cd (mg/kg diet)			
Zn (mg/kg diet)	0·16	1·5	6·1	18
30	43·5	18·2***	9·3***	6·5***
300	27·4**	17·0***	5·2***	4·9***
1000	9·5***	5·0***	5·1***	3·7***

SEM = 2·3

Caeruloplasmin was determined by the method of Houchin (1958) using the standardization of Rice (1962).
Caeruloplasmin activity of all groups is significantly lower (**$P < 0.01$, ***$P < 0.001$) than that of the control group (300 mg Zn/kg plus 16 mg Cd/kg).

Liver Cu concentrations were depressed by 47% ($P < 0.001$) in animals receiving 18 mg Cd/kg or 1000 mg Zn/kg. Cadmium accumulated in the liver and kidneys of all rats on Cd-supplemented diets. High dietary Zn concentrations reduced Cd accumulation in kidney ($P < 0.001$) but not in liver.

Addition of Cd or Zn reduced CBI. Cd at 1·5 mg/kg reduced CBI by 8·9% ($P < 0.05$) and 1000 mg Zn/kg had a similar effect.

These results indicate that a relatively small increase in Cd intake can adversely affect Cu metabolism when Cu intake is marginal.

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An individual dietary survey of schoolchildren in Birmingham. By Nicola Ruck (introduced by Joan M. L. Stephen), *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1 7HT*

A dietary survey was carried out to investigate the food intakes of children in a community where there was some evidence that rickets was occurring. Individual 7 d weighed inventory records were kept by forty-seven schoolchildren (forty-one boys and six girls), aged 14–16 years, in Birmingham. They answered questionnaires on social background and habits. The children were selected from a group of 570 who had already co-operated in a health survey (Cooke, Swan, Asquith, Melikian & McFeely, 1973). Daily intakes of nutrients (mean values and standard deviations) for the three racial groups, and for boys and girls were as follows (intakes of Asians and West Indians were compared with those of European children, and boys with girls):

Children	Energy MJ	Energy kcal	Vitamin D (μg)	Calcium (mg)	Phytate (mg)
Whole group (47)	9·07±2·55	2170±610	1·70±1·20	780±310	160±130
Asian (22)	8·95±2·93	2140±700	1·50±1·20	850±330	230±140***
West Indian (11)	8·49±2·13	2030±510	1·90±1·30	540±240*	80± 30
European (14)	9·61±2·59	2300±620	1·60±1·00	780±260	90± 40
Boys (41)	9·49±2·47	2270±590	1·70±1·20	790±310	170±130
Girls (6)	6·02±1·38	1440±330**	1·10±0·60	510±130	90± 30

Numbers of subjects in parentheses.

$*P<0·01$; $**P<0·005$; $***P<0·001$.

The vitamin D intakes were low, as found in Glasgow in a similar community (Dunnigan & Smith, 1965). There was no general relationship between serum alkaline phosphatase concentrations and vitamin D or phytate intakes, nor between vitamin D intakes and growth.

Margarine and eggs were the main sources of vitamin D, each providing about one-third of the total consumed. The West Indians ate four times as much margarine as the others, which may be one reason why this group is not prone to rickets. The Europeans had more liver and fatty fish than the others. The Asians had less of all sources of vitamin D except butter. Thirty-four of the forty-seven children drank at least 0·5 pint of milk daily.

It was not possible in this study to assess the relative exposure of different children

to sunlight. The urban environment and Moslem restrictions on women are likely to reduce the availability of non-dietary vitamin D. This, and the low dietary intakes, could be causes of the rickets and osteomalacia reported in Birmingham.

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Muscle cell size as an index of potential for growth in rats. By A. M. STEWART, *Department of Agriculture, University of Rhodesia, Salisbury, Rhodesia*

A rebound acceleration of growth after a period of retardation is a fundamental characteristic of the growth of animals. Much information is available on gross measurements such as rates of growth and voluntary intakes during the early stages of recovery from undernutrition. However, there is as yet little knowledge of relationships between these measurements and the cellular events occurring in muscle, the main tissue of the body. Such knowledge could provide further insight into the mechanisms of compensatory growth. For this reason information was sought from experiments on rats in order to formulate working hypotheses for investigations on farm animals.

In a preliminary experiment I observed that absolute increases in body-weight were similar during the first few weeks after weaning at 21 d in rats suckled in litters of three or sixteen (Stewart, 1973). Growth rates from approximately 200 g body-weight were higher in the former animals.

An experiment was designed to test the hypothesis that muscle cell size in animals at 200 g body-weight provides an index of potential for growth. Four groups of rats were reared along different growth curves to 50 g body-weight. Three groups grew directly to this weight in litters of three (group A), eight (group B), and sixteen (group C). The fourth group (D) was reared in litters of three to 75 g body-weight. Subsequently food intakes were reduced to between 1·5 and 2·0 g/d so that body-weight returned slowly to 50 g. At 50 g, all animals were fed *ad lib*. Voluntary food intakes and live-weight gains were measured as the animals approached 200 g. Results for the last 7 d are recorded in Table 1. The animals were killed at 200 g, and the vastus lateralis from the quadriceps group of muscles was dissected out for the determination of DNA and crude protein.

A highly significant inverse linear relationship was observed between protein: DNA ratios (an index of cell size; Cheek, Brasel & Graystone, 1968) in the muscle and live-weight gains (Table 1).

The similarity of voluntary food consumption in all groups revealed that energy intakes were not related to rates of synthesis of body material.

Table 1. *Protein : DNA ratios in vastus lateralis of rats in groups A, B, C and D, each of five animals, weighing 200 g, their voluntary food intakes, and gains during the 7 d before the attainment of 200 g*

	Value				LSD	
Measurement	B	A	C	D	$P=0.05$	$P=0.01$
Protein: DNA ratio	203·7	163·3	223·4	160·4	9·2	12·6
Voluntary food intake (g)	102·3	104·9	105·4	103·7	12·1	16·4
Wt gain (g)	39·2	45·7	31·6	51·4	5·6	7·6

The results also provide evidence for the conclusion that the larger the cell size in muscle tissue at a particular body-weight, the lower is the potential for further body growth.

Thanks are due to Mrs Colleen Watts and Mr E. Kumbula for technical assistance.

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The effect of an orally administered L–thyroxine supplement on reproduction in protein–deficient rats. By W. S. SOERJODIBROTO, R. J. C. STEWART and C. R. C. HEARD, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1 7HT*

Low birth weights and slow growth have been reported in a colony of rats maintained for seven generations on a diet marginally deficient in protein (Stewart, 1972). Protein deficiency is known to cause endocrine disturbances (Heard & Stewart, 1971; Rao, Kamala, Raghuramulu & Srikantia, 1971), and the possibility of slow growth and hormonal changes being related was investigated.

Twenty-one pregnant rats of the seventh generation of the low-protein colony (ratio, energy supplied by utilizable protein: total metabolizable energy=0·068) were available and thirteen were given orally a daily supplement of 0·1 μg L-thyroxine during the last third of gestation and for the first 21 d *post partum*.

Weights at conception averaged 174 g for the thyroxine-treated ($n=13$) and 166 g for the control ($n=8$) group. The difference was not significant. Litters were slightly larger in the untreated group, averaging 7·88 pups compared with 6·77 pups from the treated mothers, but this difference also failed to reach statistical significance.

Mean birth weight was increased from 4·9 g in the untreated groups ($n=63$) to 5·4 g in the thyroxine-treated group ($n=86$), a value which is equal to that found in the well-fed rat colony. This difference, in contrast to those for weights at conception and numbers per litter, was highly significant ($P<0.001$).

A similar test carried out with members of a well-fed colony had little, if any, effect; the mean birth weight after thyroxine treatment was 5·4 g, that is the same as the treated deficient animals and the untreated control colony.

Thyroxine treatment of the deficient mothers during suckling had a slight effect on the growth of the young, which remained at about 40% of normal weight.

Further experiments are planned to determine whether the thyroxine effect is mediated by changes in the food intake or by adjustments in maternal metabolic processes.

These tests indicate that, in animals suffering from a long-term marginal protein deficiency, physiological doses of thyroxine can modify intra-uterine growth.

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Effects of early undernutrition on avoidance learning in mice. By P. D. LEATHWOOD and J. MAURON, *Nestlé Products Technical Assistance Co. Ltd, Research Laboratories*, 1814 *La Tour de Peilz, Switzerland*

Many workers have shown that early undernutrition in animals can lead to permanent physical stunting, long-lasting changes in food-related behaviour and decreased exploratory activity. However, no consistent trends have yet emerged in attempts to measure learning (Dobbing & Smart, 1973). Shuttle-box avoidance learning has been applied with considerable success to the study of behavioural genetics in mice (Bovet, Bovet-Nitti & Oliverio, 1969). We have shown that early undernutrition produces permanent impairment of avoidance-learning performance in Swiss white mice (Leathwood, Bush & Berent, unpublished results).

Here we compare the effects of two different techniques for inducing early undernutrition: (a) rearing pups in large litters and (b) feeding the mothers on a low-protein diet. Pregnant mice were given a diet containing 50 g protein/kg from the 10th day of gestation until birth and a diet containing 100 g protein/kg during lactation. Controls were given a diet containing 200 g protein/kg throughout gestation and lactation. At birth all the pups were cross-adopted to give the following experimental groups.

Control: born and reared (five/litter) by well-nourished dams.

'20's': born and reared (twenty/litter) by well-nourished dams.

Pre-natally undernourished: born of protein-restricted dams, reared by well-nourished ones.

Post-natally undernourished: born of well-nourished dams, reared by protein-restricted ones.

Pre- and post-natally undernourished: born of, and reared by, dams on protein-restricted diets.

All mice, except the '20's' were reared in litters of five pups. Patterns of physical and behavioural development were studied throughout the suckling period. The mice were weaned at 21 d when all experimental groups exhibited some degree of physical stunting compared with the controls (Table 1).

Table 1. *Weaning and adult weights of previously undernourished mice expressed as percentage of that of the controls*

Group	Weaning wt	Adult wt
Control	100	100
'20's'	40·6	78·3
Post-natally undernourished	66·2	75·7
Pre-natally undernourished	47·5	84·9
Pre- + post-natally undernourished	41·3	71·4

From 21 d all mice were fed *ad lib*. on stock diet and avoidance-learning performance (fifteen daily sessions of fifty trials) was measured from 9 weeks on. The results are given in Table 2.

Table 2. *Avoidance performance of previously undernourished mice*

Group	No. of mice	Mean percentage of successful avoidances with SEM
Control	16	50·8 ± 3·3
'20's'	22	22·7 ± 6·4*
Pre-natally undernourished	13	29·7 ± 5·8*
Post-natally undernourished	12	38·4 ± 4·5*
Pre- + post-natally undernourished	21	29·1 ± 4·2*

*Significantly less than value for controls ($P < 0.025$).

Early undernutrition produced either by malnourishing the mother or by rearing in large litters significantly impaired avoidance-learning performance. Mice raised in litters of twenty achieved the least successes and were also the most severely stunted at weaning. When pups were reared by malnourished mothers, deprivation for 10 d before birth had more pronounced effects on performance than under-nutrition for the 21 d from birth to weaning.

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The effect of oral administration of sodium phenobarbitone on nitrogen balance in the rat. By DELIA M. FLINT and D. P. RICHARDSON, *Department of Nutrition, Queen Elizabeth College, London W8 7AH.*

Many illnesses and stress situations are treated by the administration of drugs which can interfere with the availability and utilization of certain nutrients.

In a pilot study sodium phenobarbitone was given to thirteen rats and its effect on

nitrogen balance was measured. The food intake was kept constant throughout three successive periods of 5 d. During period 2, the drug was given in the diet as a daily dose of 100 mg/kg body-weight; in periods 1 and 3 the diet alone was given. Table 1 summarizes the results. During the first period a positive balance was observed.

Table 1. *Mean values (mg/d) for nitrogen excretion and balance in groups of thirteen rats given a constant food intake alone (days 1–5 and 11–15) or with a daily addition of 100 mg phenobarbitone/kg body-weight (days 6–10)*

Days	Urinary N	Faecal N	N Balance
1– 5	473	41	÷78
6–10	541	39	−14
11–15	504	42	+18

When the drug was given a negative balance resulted. When the drug was withdrawn positive balance was restored after 2 d. Daily analysis of the urine revealed that the negative nitrogen balance was due to a significant increase in the excretion of urinary nitrogen ($P = 0.01$).

The FAO/WHO (1965) Expert Committee on Protein requirements recognized that the stresses of everyday life have a marked effect on nitrogen metabolism. As barbiturate drugs are widely used for the treatment of sleeplessness and nervous disorders, they could act either alone or in combination with an underlying medical condition to further affect the nitrogen requirement of an individual. Giving the drug in the diet proved to be a convenient technique because in most experiments it is given by intraperitoneal injection, which would have been an additional stress. In the present study the effects are attributed to the barbiturate drug alone.

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