The Two Hundred and Fifty-second Scientific Meeting of the Nutrition Society was held in Guy's Hospital Medical School, St Thomas Street, London SE1 9RT, on Thursday, 7 December 1972, at 14.00 hours, when the following papers were read:

The effect of various carbohydrates on weight gain and carcass composition in the rat. By A. R. MacRae*, S. J. Slinger, Irene Nickel and the late T. S. Neudoerffer, *Department of Nutrition, University of Guelph, Guelph, Ontario, Canada*

In view of the fact that different carbohydrates have been observed to have different effects on body-weight gain and carcass composition (Marshall, Womack, Hildebrand & Munson, 1969), a study was initiated to observe the effects of two dietary levels of sucrose, glucose, fructose, and maize starch on the weight gain and carcass composition of rats.

Fifty-four young male rats (150 g) of the Wistar strain were given modifications of a basal diet (MacRae & Neudoerffer, 1971) the composition of which (g/kg) was: casein 300, methionine 10, maize oil 125, maize starch 410, cellulose 75, plus the necessary vitamins and minerals. Either sucrose, glucose, fructose or maize starch was added to the basal diet so that the final mixture contained 400 or 600 g/kg of the added carbohydrate and yet still provided adequate levels of all nutrients (casein levels 180 g and 120 g/kg respectively). Groups of six rats were given the diets for 30 d with the intake controlled to that observed on the least palatable diet (400 g maize starch substituted). Carcass moisture, fat and protein content were estimated at the end of the experiment.

At both the 400 g and 600 g/kg levels of addition, the sucrose-fed animals showed the greatest weight gains followed by, in decreasing order, the groups given maize starch, glucose, and fructose. With every carbohydrate, greater weight gain occurred on the 400 g/kg diet.

The greater increase in weight in rats on both sucrose diets was reflected by an increase in the carcass fat content of these animals. In addition, the carcass protein content was highest on the diet containing 600 g sucrose/kg, and the carcass moisture content was highest on the diet with 400 g sucrose/kg. A comparison of the carcass fat contents of animals given sucrose and fructose showed similar values on the 400 g/kg diets, but lower values with fructose at the 600 g/kg level. In carcass protein content, the results obtained for fructose-fed animals were intermediate between those found with other carbohydrate diets. Animals given diets with 600 g glucose and maize starch/kg diet had similar weight gains but different carcass compositions: glucose-fed animals had less moisture and more fat in the carcass.

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On the diets with 400 g glucose and maize starch/kg diet, differences in carcass fat and moisture content were smaller, but animals on the glucose diet had significantly less carcass protein.

These results demonstrate that different carbohydrates can elicit different weight gain and carcass composition responses in the laboratory rat.

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The use of gold–thioglucose and monosodium glutamate to induce obesity in mice. By A. DJAZAYERY and D. S. MILLER, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Gold-thioglucose (GTG) and monosodium glutamate (MSG) have been used to induce obesity in adult and neonatal mice (Olney, 1969; Debons, Krimsky, From & Cloutier, 1970). The purpose of this study was to investigate the effect of GTG and MSG on growth, food intake, and carcass composition of weanling mice as well as to show whether the hyperphagia which leads to obesity is temporary or permanent.

Groups of weanling, female mice of the Ash-XP strain were injected intraperitoneally with either 0.5 mg GTG or 5 mg MSG/g body-weight. The ones that survived (50% of the former and 20% of the latter died), together with a control group, were fed on Oxoid *ad lib*. Food intake was measured throughout the experiment. After 4 months the groups were killed and the carcasses were analysed.

The results (mean values ± SEM) were:

	Final body-wt (g)	P	Carcass composition (g/kg) Water	P	Protein	P	Fat	P
GTG	56.3 ±0.9		367 ±4		95 ±1		513 ±19	
		0.01		0.01		0.03		0.03
Control	30.7 ±3.6		626 ±24		169 ±12		149 ±40	
		0.20		0.05		0.001		0.02
MSG	32.8 ±1.8		574 ±14		132 ±21		263 ±10	

These results show that GTG produced heavier animals with a higher carcass fat content. However, whereas the rate of body-weight gain was not affected by MSG, the carcass fat content was increased to about twice that of the controls after 4 months. It should be noted that the results mask individual variation. Not all the treated animals became obese. If obese animals are required for further work, animals successfully treated with GTG can be selected on the basis of body-weight, but this does not apply to MSG-treated animals.

During the 1st month the mean daily food intake (expressed as MJ metabolizable energy/$W^{0.75}$) of the GTG group was 35% more than that of the controls, whose daily intake was the same as the intake of the MSG group (0·901 MJ/$W^{0.75}$). After the 1st month, however, all groups were eating about 0·754 MJ/$W^{0.75}$ daily, which further decreased to 0·670 by the 3rd month, and stayed constant after that. During these periods all groups were gaining weight.

Thus the GTG-induced obesity appears to be due to an initial hyperphagia but, since the food intake soon returns to control values, the continued development of obesity must be due to a change in metabolic efficiency. In the MSG animals, where the food intake is always equal to the intake of their lean controls, the obesity must be entirely due to a change in energetic efficiency. The effects of these treatments on energy metabolism are the subject of the following paper (Djazayery, Miller & Stock, 1973).

We wish to acknowledge the technical assistance of Mr R. Cox.

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Energy balances of mice treated with gold–thioglucose and monosodium glutamate. By A. DJAZAYERY, D. S. MILLER and M. J. STOCK, *Department of Nutrition and Department of Physiology, Queen Elizabeth College, London W8 7AH*

In this paper we report on some of the measurements of energy expenditure that have been made on mice treated with gold-thioglucose (GTG) and monosodium glutamate (MSG) as described in the previous paper (Djazayery & Miller, 1973).

Measurements of the energy expenditure of groups of four mice were made every 24 h, using a gravimetric system for estimating oxygen consumption and carbon dioxide production according to the method devised by Haldane (1892). One group from each of the two treatments plus a control group were studied during the 2nd and 3rd months after injection of GTG or MSG.

The mean values for daily energy expenditure ($n=10$ for each group) over this period were 0·57, 0·53 and 0·44 MJ/$W^{0.75}$ for the control, MSG and GTG groups respectively. The lower values for the two treated groups are significantly different from the control values (MSG, $P<0.05$; GTG, $P<0.00001$).

During the 3rd month after injection, food intakes were also measured and these values together with the simultaneously measured energy expenditures are shown in Table 1, as well as the final average carcass composition of the mice.

It should be noted that, although the GTG animals as individuals ate more than

Table 1. *Daily energy balances and carcass composition of mice treated with mono-sodium glutamate (MSG) or gold-thioglucose (GTG)*

	Control (4)[*]	MSG (3)[*]	GTG (5)[*]
		MJ/mouse	
Intake	0·043	0·041	0·062
Expenditure	0·042	0·037	0·048
Balance	0·001	0·004	0·014
		$MJ/W^{0.75}$	
Intake	0·599	0·629	0·566
Expenditure	0·587	0·570	0·453
Balance	0·012	0·059	0·113
		Final carcass composition	
Body-wt (g)	30·2	28·8	53·3
Fat (g/kg)	187	180	448

[*]Number of measurements.

the controls, when expressed as a function of metabolic body-weight ($W^{0.75}$) their energy intake was lower than the control value. This is also true if the intakes are expressed as a direct function of body-weight.

Thus the continuing increase in the degree of obesity during this period cannot be ascribed to GTG-induced hyperphagia, since in metabolic terms these animals eat less than their lean controls. The greater energy retention of the GTG mice appears to be related to their lower energy expenditure, and this is also partly true for the MSG group. The results for the latter group are difficult to interpret, since the final carcass analysis revealed that only two out of the four MSG-treated animals had deposited more fat than the controls.

An index of the feed conversion efficiency is obtained by expressing the energy retained as a fraction of the energy intake. Values of 0·02, 0·09 and 0·22 are obtained for the control, MSG and GTG groups respectively, indicating the greater metabolic efficiency of the treated animals, which results in their becoming obese.

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The effects of diethylstilboestrol on obesity in mice. By A. DJAZAYERY and D. S. MILLER, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Diethylstilboestrol (DES) has been reported to depress growth and food intake in rats (Meites, 1949) and reduce growth in mice (Saxena & Pal, 1967). The present study was conducted to investigate the effect of DES on the growth, food intake, and

carcass composition of obese mice. Weanling female mice of Ash-XP strain were injected with gold-thioglucose (GTG, 0·5 mg/g body-weight) or monosodium glutamate (MSG, 5 mg/g body-weight) and fed on Oxoid diet *ad lib.* Also, a group was fed on a high-protein–high-fat (HF) diet (Miller & Parsonage, 1971). After 7 weeks DES was added to the diet (2 mg/kg diet) of one group of each of the GTG, MSG and HF groups; a group of each continued on the previous diets to serve as controls. The food intake was measured during the 3rd week of the experiment, and all groups were killed and analysed after 4 weeks. The results were:

	Daily food intake*	Body-wt (g)		Final carcass composition (g/kg)		
		Initial	Final	Water	Protein	Fat
GTG-DES	0·478	49·0	34·6	589	146	242
GTG-control	0·727	49·0	56·7	468	112	405
MSG-DES	0·670	27·5	26·5	686	147	147
MSG-control	0·637	27·5	30·4	602	164	222
HF-DES	0·557	33·7	27·7	655	166	157
HF-control	0·645	33·7	36·1	453	220	293

*MJ metabolizable energy/$W^{0.75}$.

All groups treated with DES lost weight and fat. The weight lost by the treated MSG group was small but their fat loss was similar to that in the other groups. During the experiment, the food intakes of the GTG, MSG and HF groups were 66, 105 and 86% of their non-treated controls respectively.

These findings indicate that diethylstilboestrol causes loss of fat in obese mice. In those mice made obese with GTG or with a high-fat diet, there is a reduction in food intake, but in mice made obese with MSG food intake is unaffected. Losses of protein were small compared with losses of fat.

We wish to acknowledge the technical assistance of Mr R. Cox.

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Relationships between sex hormones and fructose metabolism in rat livers. By A. W. M. HAY and J. B. PRIDHAM (introduced by I. MACDONALD), *Department of Biochemistry, Royal Holloway College, University of London, Englefield Green, Surrey*

Studies in vivo suggest that the rate of metabolism of fructose and in particular the conversion of this hexose into plasma triglyceride is sex-dependent in some animals (cf. Macdonald, 1966; Jourdan, 1969; Hill, 1970).

A study has been made of various measurements affecting the metabolism of [^{14}C]fructose by rats with special reference to liver ketohexokinase (*EC* 2.7.1.3: ATP:D-fructose 1-phosphotransferase) activity.

The specific activity of ketohexokinase was high in tissues from 3-d-old animals and decreased sharply at 10 d. A further but less marked lowering of activity occurred with increasing age, and with sexually mature animals (9–11 weeks old) the specific activity in male liver tissues was approximately twice that found in female tissues.

Intraperitoneal injections of sex hormones into mature rats produced differences in the rate of fructose phosphorylation by liver-tissue preparations. Thus, testosterone gave rise to increases in both male and female animals whereas oestrone appeared to lower the ketohexokinase activity in male rat tissues and increased it in female tissues. Similar results were obtained when liver slices were incubated with the hormones.

No effect on liver ketose-1-phosphate aldolase (ketose-1-phosphate aldehyde-lyase:EC 4.1.2.7) activity was observed when rats were injected with testosterone. However, the specific activity of this enzyme in the livers of normal, mature, male rats was double that found in female tissues.

Sex-linked differences in the production of α-glycerophosphate from fructose have also been observed.

The relevance of these observations in relation to plasma triglyceride formation was discussed.

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Some effects of diet with oral contraceptives on carbohydrate:lipid metabolism in the baboon. By VALERIE STOVIN and I. MACDONALD, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

It has been found that dietary sucrose produces an increase in the fasting serum triglycerides of men and post-menopausal women but not of pre-menopausal women (Macdonald, 1967). There is also evidence that the oestrogen-containing oral contraceptive affects lipid metabolism, as shown by the rise in the fasting serum triglyceride concentration (Stokes & Wynn, 1971). It seemed worth while, therefore, to learn whether a sucrose diet given together with a hormonal contraceptive augmented the serum lipid rise associated with this method of contraception.

Six sexually mature female baboons were subjected to dietary regimens lasting for 7 weeks. The composition (g/kg) of the experimental diets was: sucrose or glucose 750, calcium caseinate 180, dried yeast 50, salts 20, and added vitamins. The dietary mixture was made up in water and the amounts given were sufficient to keep the weights of the animals constant. The diets, administered in random order, were given either with or without an oral contraceptive (1·0 mg norethisterone and 0·05

mg mestranol); another regimen consisted of giving the normal laboratory diet with the oral contraceptive.

Fasting venous blood samples were taken before each diet began and after 3 and 7 weeks on the diet. The serum was examined for cholesterol and triglyceride concentrations.

Though increases were found in the mean triglyceride concentration of fasting serum on days 21 and 49 of each regimen, only the increase on day 49 of the sucrose diet plus oral contraceptive was statistically significant. No significant changes were found in the fasting serum cholesterol concentrations.

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The effect of intermediates on the incorporation of fructose and glucose into hepatic triglyceride in rats. By Monica Wusteman and I. Macdonald, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

Fructose, when compared with glucose, makes a greater contribution to glyceride formation in the liver of rats (Nikkila, 1966), and largely to the glycerol moiety (Bar-on & Stein, 1968). These findings, when taken together with the observation that glycerol causes a significant rise in serum triglyceride concentration in men (Macdonald, 1970), suggest that the greater contribution of fructose to triglyceride formation could be by an increase in the production of α-glycerol phosphate. It was therefore decided to give to rats [14C]fructose with and without glycerol. As the trioses, glyceraldehyde and dihydroxyacetone phosphate, are intermediates in fructose metabolism, these substances were also given with [14C]fructose (the dihydroxyacetone was given without the high-energy phosphate).

After an overnight fast, young, male, Wistar rats (200 g) were given, intragastrically, [14C]fructose either alone or with glycerol, glyceraldehyde or dihydroxyacetone. Glucose alone and with glycerol were also given. The animals were killed 2 h later and the livers removed. After extraction of the lipids from the liver, the triglyceride was separated by thin-layer chromatography and the radioactivity was determined.

The results showed a striking rise in radioactivity in the liver triglyceride when glycerol was given with the [14C]fructose compared with when the [14C]fructose was given alone. Dihydroxyacetone reduced the specific activity in liver triglyceride, and after glyceraldehyde was given the specific activity was similar to that when fructose was given alone. When compared with [14C]glucose given alone, the addition of glycerol to [14C]glucose resulted in an increase in liver triglyceride specific activity.

Thus from these results it would appear that the increased propensity of fructose to form serum triglyceride is not, in rats, due simply to its greater ability to provide higher concentrations of α-glycerol phosphate.

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The effect of high-carbohydrate-low-energy diets on body-weight in man. By I. MACDONALD and JOCELYN TAYLOR, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

It has been shown that rats and baboons given a diet with a high content of sucrose have a greater increase in body-weight and body fat than when the sucrose is replaced by a partial hydrolysate of starch or fructose in amounts of equal energy (Allen & Leahy, 1966; Brook & Noel, 1969). To see whether changes in body-weight in man were dependent on the type of carbohydrate eaten, six men and four women (20–25 years of age) were given formula diets containing approximately 10% less energy given that required for energy balance. The diet consisted of calcium caseinate (1 g/kg body-weight) and either sucrose (6·5 g/kg body-weight) or partial hydrolysate of starch (6·5 g/kg body-weight) made up to 20 ml/kg body-weight with water. The diets were given by stomach-tube two to three times daily for a period of 11 d. A vitamin tablet (BPC) was taken each day and the subjects were allowed to drink black coffee and water but take no other food.

Five subjects were given the high-sucrose diet first and there was an interval of several weeks between change of diets to allow the body-weight to stabilize.
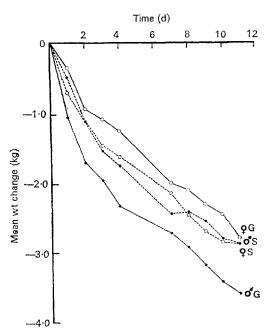

Fig. 1. Mean weight loss in men and women on low-energy diets containing equal amounts of partially hydrolysed starch or sucrose. S, sucrose; G, partially hydrolysed starch.

Weight loss tended to be greater while on the hydrolysed starch diet than while on the sucrose diet, but when the findings for each sex were considered separately it was found that the men lost more weight while on the partially hydrolysed starch diet ($P < 0.001$) and the women lost more weight while on the sucrose diet ($P < 0.001$). The total weight loss while on the sucrose diet was the same for both sexes (Fig. 1).

We are very grateful to the subjects.

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Polyunsaturated fatty acids in plasma lipids of cord blood in newborn babies. By BRIAN WHARTON and AUDREY FOSBROOKE, *Institute of Child Health, Guilford Street, London WC1N 1EH*

The fatty acid composition of plasma cholesterol ester and triglyceride has been determined in the cord blood of nineteen term, sixteen pre-term and fourteen light-for-dates babies. The results, expressed as g/kg total fatty acid $\pm$ SD, were:

		Pre-term babies		Term babies		Light-for-dates babies
Cholesterol ester:	linoleic acid	130 ± 30	$P < 0.001$	170 ± 40	NS	160 ± 50
	arachidonic acid	80 ± 20	$P < 0.001$	110 ± 30	NS	110 ± 40
Triglyceride:	linoleic acid	70 ± 30	$P < 0.001$	110 ± 20	NS	100 ± 40

The proportion of polyunsaturated fatty acids was significantly lower in the pre-term group than in the term babies. In the light-for-dates babies the values were similar to those in the term group.

The proportion of polyunsaturated fatty acids in the plasma lipids of cord blood is influenced by the maturity of the foetus but is independent of its nutritional status.

The diurnal variation in volatile fatty acids concentration in portal and atrial plasma in pigs. By D. M. ANDERSON and A. J. NORTHROP, *Department of Biochemistry, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

Previous studies of volatile fatty acids (VFA) in portal plasma in pigs have been confined to spot samples from anaesthetized animals undergoing surgery (Barcroft, McAnally & Phillipson, 1944; Friend, Nicholson & Cunningham, 1964). In the present experiments serial blood samples were taken through indwelling catheters from the portal vein and right atrium of unanaesthetized pigs at least 7–10 d after surgery. The pigs weighed 25-40 kg and were given pellets (g/kg: crude protein 190, crude fibre 55) at 40 g/kg body-weight daily in one or two feeds. VFA concentration was determined by freeze-transfer and gas–liquid chromatography (P. J. Barker and D. B. Lindsay, unpublished).

Table 1. *Concentrations of volatile fatty acids in portal and atrial plasma of pigs*
(Mean values with their standard errors)

		Concentration in plasma (mmol/l)						
		Portal						Atrial acetate
No. of feeds	Time of sampling after feed (h)	acetate		propionate		butyrate		
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	Prefeed	0·41	0·03	0·13	0·02	0·04	0·01	0·16	0·05
Fed at 09.30	5	0·42	0·02	0·14	0·02	0·04	0·01	0·20	0·03
hours B.S.T.	8	0·74	0·09	0·24	0·04	0·08	0·02	0·35	0·05
	12·5	0·66	0·1	0·26	0·05	0·09	0·01	0·32	0·04
	14	0·43	0·08	0·16	0·04	0·05	0·01	0·25	0·04
	17	0·59	0·1	0·22	0·03	0·07	0·02	0·20	0·06
2	Prefeed	0·87	0·2	0·28	0·06	0·05	0·02	0·24	0·04
Fed at 09.30	1	0·49	0·08	0.15	0·06	0·02	0·01	0·26	0·04
hours B.S.T.	6	0·87	0·15	0·34	0·13	0·05	0·01	0·40	0·02
Fed at 16.00	11	0·62	0·02	0·27	0·05	0·03	0·01	0·51	0·08
hours B.S.T.	14	0·81	0·07	0·29	0·09	0·05	0·02	0·40	0·06
	20	0·64	0·07	0·27	0·00	0·03	0·01	0·36	0·15

The effects of feeding are summarized in Table 1. In four animals fed once, VFA concentration did not alter until 5 h after feeding, then rose to a peak at 8 h, remained at this level until 12.5 h, dropped at 14 h rose again at 17 h and then declined until feeding. Propionate and butyrate were present in traces in atrial plasma, as was isobutyrate which achieved significant concentrations (0·13 mmol/l) only in the one castrated male in the series. In the three animals fed twice, portal VFA declined during the 1st hour after feeding, then rose steadily until 6 h. The second feed was given after 6·5 h and VFA decreased until 11 h; they increased again until 14 h, declined slightly until 20 h and then increased again until feeding. Atrial acetate increased slowly after the first feed to a peak at 11 h and then declined. In a fourth pig, fed twice, the tip of the catheter lay in the portal vein 50 mm caudal to the junction with the gastrosplenic vein and not in the liver, as were the other catheters. The result was a similar pattern to that described, but acetate concentrations of 2·8 mmol/l were reached. This might suggest laminar blood flow in this area of the portal vein.

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Organic phosphates in the diet as possible protective agents against dental caries in the rat. By T. H. GRENBY, *Department of Oral Medicine and Pathology, Guy's Hospital, London SE1 9RT*

One means proposed for combating dental caries is by the addition of certain phosphate compounds to foods and drinks. A wide range of inorganic phosphates has been tested, but less information is available on the organic phosphates. Three

compounds in this group were therefore tested in a series of experiments using over 400 rats: (1) sodium phytate, the sodium salt of *myo*-inositol hexaphosphate; (2) calcium sucrose phosphate, marketed as Anticay; and (3) calcium glycerophosphate.

Matched groups of caries-prone strains of rats were fed on high-sugar cariogenic diets for periods of 5–8 weeks from weaning. The organic phosphates were administered by three different methods: incorporation in the diet, addition to the drinking-water, or direct application on to the tooth surface three times per week. At the end of the trials the mandibular molar teeth were examined for caries as measured by caries scores, counts of gross cavities, and 'smooth-surface' caries scores, compared with untreated control groups.

Calcium sucrose phosphate either at 20 g per kg diet or drinking-water, or as a 75 g/l solution applied topically, had no significant effect on the incidence or severity of caries.

Sodium phytate at 10 or 20 g/l in the drinking-water had no significant effect, but at 20 g/kg in the diet or as a 200 g/l solution applied topically, it showed evidence of a small but significant protective effect in three out of four trials.

Calcium glycerophosphate did not have a significant effect when added to the drinking-water at a level of 17 g/l, but was by far the most protective compound when given as a dietary supplement at 20 or 40 g/kg, or applied topically as a saturated solution. In a series of four controlled trials it significantly reduced the caries score in all four, the number of gross cavities in three out of four, and the 'smooth-surface' caries score in two out of four trials.

The results show that the order of effectiveness against dental caries is: (1) calcium glycerophosphate; (2) sodium phytate; and (3) calcium sucrose phosphate. The wide variation in the degree of protection against dental caries afforded by the different phosphates shows the need for further work to elucidate their mechanisms of action.

Changes in the dental plaque after eating sweets containing starch hydrolysates instead of sucrose. By T. H. GRENBY and JANET M. BULL, *Department of Oral Medicine and Pathology, Guy's Hospital, London SE1 9RT*

Sucrose has been observed to cause more dental caries than other dietary carbohydrates in experimental animals. It has been suggested that the deposition of dental plaque on the teeth precedes the development of caries, and that sucrose is detrimental because it serves as a better substrate than other sugars for copious plaque formation.

As sweets and other confectionery can provide a considerable proportion of dietary sucrose, the influence of sucrose in sweets on dental plaque formation and composition was investigated. Two types of boiled sweets were compared: one a control sample with 600–800 g sucrose/kg and 200–400 g glucose-syrup solids/kg as sugar components; and the other an experimental sample in which all the sucrose was replaced by spray-dried wheat-starch partial hydrolysates, with a D-glucose content of 400–600 g/kg and a maltose content of 50 g/kg.

Equal groups of student volunteers sucked 454 g each of one or other of the samples of sweets over a 3-d period in addition to their normal diet. Their teeth were scaled and polished at the start, and they were asked not to brush them during the 3 d of the experiment. At the end of this period, plaque on the teeth was stained with disclosing solution and photographed. Then the plaque was scraped off the teeth and dried to constant weight, and each individual's plaque was analysed separately.

The results showed a positive correlation between the dental caries experience of an individual and the extent of plaque covering the buccal and labial surfaces of the teeth.

The extent of the plaque was significantly greater after the sucrose-containing sweets than after the non-sucrose sweets. From a range of small-scale colorimetric chemical analyses, it was observed that the proportion of total carbohydrate in the plaque was significantly higher after the sucrose sweets than after the non-sucrose sweets. Most of the carbohydrate appeared to be in the form of α-1,6-polyglucose, but traces of glucose and sucrose, and fructose formed from levans, were also detected. Amounts of protein, calcium and phosphorus in the plaque were not significantly influenced by the carbohydrate composition of the sweets.

The results show that a high intake of sucrose-containing sweets increased plaque formation and the proportion of carbohydrate in the plaque. These were significantly lower when all the sucrose in the sweets was replaced by wheat-starch hydrolysates.

The effect of alcohol (ethanol) on the oxygen consumption of fed and fasting subjects. By ANNE L. STOCK, M. J. STOCK and JEAN A. STUART, *Department of Nutrition and Department of Physiology, Queen Elizabeth College, London W8 7AH*

Investigations into the effect of ethanol (alcohol) on metabolic rate in man appear to have given varying and contradictory results. Barnes, Cooke, King & Passmore (1965) found that alcohol had no specific dynamic action (SDA) in man, whereas Perman (1962) had previously demonstrated an increased oxygen utilization by man after the ingestion of alcohol. However, the subjects used by Barnes *et al.* were fasting whereas Perman had not enforced this condition and it could be that the responses found in the latter study were due to an interaction between the metabolism of alcohol and the residual SDA of the previous meal.

It was therefore decided to investigate the effect of alcohol, when taken as a sole nutrient and when taken as part of a meal, on oxygen consumption in man.

Fasting subjects (five male, five female) were given three types of meal, A, B and

C, on successive days in that order, and on subsequent days the procedure was repeated in the reverse order. Meal A consisted of whisky providing o·84 MJ, meal B was a 2·93 MJ breakfast which included o·84 MJ sucrose, and meal C was the same breakfast but with o·84 MJ whisky replacing the sucrose. The meals were all consumed in 10 min. Oxygen consumption was measured with the Benedict–Roth spirometer in the fasting state (four recordings) and serially for a total of 95 min postprandially (seven recordings).

The mean results for the ten subjects are shown in Table 1 and it can be seen that, in accord with the results of Barnes *et al.* (1965), the drinking of alcohol (A) causes only a small and non-significant increase in postprandial oxygen consumption. However, when taken with food (C) the effect of alcohol is almost to double the thermogenic response normally obtained with a non-alcoholic meal of equal energy value (B).

Table 1. *Mean oxygen consumption (ml/min, standard temperature and pressure, dry) of ten subjects given meal A, B or C*

	A	B	C
Preprandial	227	229	230
Postprandial	232	258	280
	$P > 0.05$	$P < 0.01$	$P < 0.01$
$\triangle V_{O_2}$	5	29	50
	$P < 0.01$		$P < 0.01$

It should be noted that the increase in oxygen consumption produced by meal C is even greater than the sum of the increments produced by meals A and B (equivalent to feeding with 3·77 MJ). In general, it was found that the magnitude of the response to all three meals was lower in females than in males.

We thank all those who volunteered as subjects.

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The effect of age on the thermic energy of 'catch–up' growth. By D. S. MILLER and A. WISE, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

After a period of restricted feeding, animals when refed grow faster than if they had not been restricted. In this experiment the efficiency of food utilization was measured during the period of 'catch-up' growth. Animals were maintained at a constant weight, either by a low-protein diet or by restricting the intake of a stock diet. The period of weight maintenance lasted for 1 month, after which the efficiency of growth over a 6-d refeeding period was assayed by the comparative carcass method

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of Miller & Stock (1969). Untreated animals of the same weight served as controls. The experiment was carried out in duplicate with groups of four hooded rats of either 3 or 5 weeks of age. The results were:

Age of rats at start of assay (weeks)	Previous diet	Metabolizable food intake	Carcass gain	Thermic energy*	Thermic energy: metabolizable energy ratio
		(kJ/rat per 6 d)			
3	Stock	674	171	210	0·31
		770	226	270	0·35
7	Stock restricted	973	380	217	0·22
		1170	496	189	0·16
7	Low-protein	802	265	182	0·23
		948	301	223	0·24
5	Stock	1050	272	215	0·20
		1000	221	235	0·23
9	Stock restricted	1570	517	407	0·26
		1560	491	436	0·28
9	Low-protein	1250	231	398	0·32
		1380	416	380	0·28

*Metabolizable energy—carcass gain—0.45 $MJ/W^{0.75}$.

After the period of weight maintenance the treated animals grew faster than the controls. The rats previously on restricted stock diet grew faster than those previously fed on a low-protein diet, but both these groups had equivalent thermic energies. It was found that when weight maintenance began at 3 weeks of age, the thermic energy of 'catch-up' growth was lower than it was for the controls, whereas in the older rats the thermic energy was greater than for the controls.

The literature contains reports that 'catch-up' growth is sometimes more efficient (Meyer, Lueker & Smith, 1956; Mahendra & Miller, 1969) and sometimes less efficient (Ashworth, Brook & Waterlow, 1972). These experiments indicate that the thermic energy of 'catch-up' growth depends on the age of the animals, which is consistent with the findings of Widdowson & McCance (1963) on food conversion efficiency.

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The repeatability of a questionnaire on the dietary intake of sugar, designed for self-administration. By J. F. RICHARDSON (introduced by G. L. S. PAWAN), *Institute of Directors (BUPA) Medical Centre*, 210 *Pentonville Road, London N*1

Yudkin's (1967) questionnaire on the dietary intake of sugar, designed for self-

administration was used to assess the sugar intake of a group of 415 businessmen (Richardson 1972), as part of a study of various risk factors for ischaemic heart disease. The results showed that factors such as age, relative weight (actual weight as a percentage of expected weight for height and age), cigarette smoking, and adequate exercise were statistically correlated with the sugar intake. In addition, the statement that 'sugar was restricted' was shown to be a factor of considerable importance affecting the reported level of sugar intake.

The possible role of sucrose as a risk factor in ischaemic heart disease (Medical Research Council Working-party, 1970) makes it necessary that a commonly used questionnaire for the dietary intake of sugar should produce repeatable results over a period of time.

A copy of Yudkin's questionnaire was posted, together with a covering letter explaining the purpose of the request and a stamped addressed envelope, to fifty-six randomly selected subjects in November 1970, just 2 years after the original inquiry.

Forty-two subjects replied to the first letter (75%) and a further three to a second request, giving a total response rate of 80·4%, and a 10·8% sample of the original group. One questionnaire was excluded from the analysis as the subject was one of the eight subjects who failed to complete the original questionnaire.

The only significant difference in the subjects' mean sugar intakes over the 2-year period occurred in group 2—that is those subjects who previously were not restricting their sugar intake but who now admitted to doing so (Table 1). The correlation

Table 1. *Mean sugar intake of groups of businessmen according to change in sugar restriction*

Group no.	Initial status (Oct. 1968)	Repeat status (Nov. 1970)	No. of subjects	Mean sugar intake (g/week)				Paired comparison of results
				Initial		Repeat		
				Mean	SD	Mean	SD	
1	o	o	12	477	191	599	252	NS
2	o	R+	12	418	196	363	172	0·025 > P > 0·01
3	R+	o	1	614	0·0	486	0·0	—
4	R+	R+	19	193	203	184	205	NS
Total			44	342	234	353	267	NS

R+, admits restricting sugar; o, denies restricting sugar; NS, not significant.
Difference between means for groups 1 and 4, on initial result, $P < 0.005$.
Difference between means for groups 1 and 4, on repeat result, $P < 0.005$.

coefficient between the sugar intake on the first and second occasions for those thirty-one subjects who did not change their status regarding sugar restriction (groups 1 and 4) was also significant ($r = 0.86$, $P < 0.001$).

The particular advantage of this questionnaire is the avoidance of observer bias, and the repeatable results obtained support Walker's (1971) belief in the general validity of information on sugar consumption obtained by this method.

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Dietary pattern of carbohydrate provision and accident incidence in foundrymen. By J. D. BROOKE and S. TOOGOOD, *Human Performance Laboratory, Physical Education Section, University of Salford,* and L. F. GREEN and R. BAGLEY, *Beecham Products, Beecham House, Great West Road, Brentford, Middlesex*

The workers' metabolic load in industrial production is important. Disequilibrium in oxygen provision or availability of nutrients leads to fatigue, errors of performance or absenteeism. We report preliminary information on work performance and nutrition in foundrymen.

In the first study on three subjects, oxygen uptakes $\dot{V}o_2$, respiratory quotients and heart rates were obtained by the procedure described by Brooke & Davies (1970, 1972). With mean $\dot{V}o_2$ of 988 ml/min and mean heart rates of 103 beats/min, oxygen provision was adequate for an habitual industrial task. Non-protein respiratory quotients in the early mornings were low, and the three subjects ate little food before work; low-carbohydrate availability appeared to be associated with accident incidence. There were more morning than afternoon accidents, and more occurred before the morning-break than after it.

In the second study, over a 3-d period, twelve factory workers were given three dietary treatments, according to a Latin square design, each in addition to their normal diet. The treatments were: glucose syrup drinks (two presentations of 250 ml, each of 1486 kJ energy value, in the first session), a low-energy drink in identical volumes ($<$20 kJ/250 ml), and no treatment. Dietary surveys were made and respiratory quotients were measured.

The normal respiratory quotient pattern was similar to that of the first study; the respiratory quotients revealed a boost in available carbohydrate with the glucose syrup supplement in comparison with the normal diet. Noteworthy was an initial rise that occurred with the low-energy fluid, although this experimental effect dissipated later in the day to result in values lower than normal, even though nutrition was otherwise normal. Dietary surveys revealed marked intra-day imbalance with little nutrient taken before the mid-morning break. There was no differentiation between dietary treatments in the oxygen uptake of subjects, mean values being very close to those of the first study.

Very recently an 18-week study has been made of accidents and physiological conditions in approximately seventy foundrymen who were provided with one of the two treatments each morning. Every 4–6 weeks men changed to the other treatment, thus acting as their own controls. In addition, a group of seventeen men, who did not wish to take either drink, was studied. Accidents, respiratory quotients, blood sugars, and daily diets for the three groups are being analysed. Preliminary

tabulation of the accidents shows that fewer accidents occurred with the glucose syrup treatment than with either the low-energy treatment or the non-co-operating group. The correlation earlier observed between low-carbohydrate availability and accidents in workers appears to be soundly based.

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Total body fat, calculated from body density, and its relationship to skinfold thickness in 571 people aged 12–72 years. By J. V. G. A. DURNIN and J. WOMERSLEY, *Institute of Physiology, The University, Glasgow W2*

Measurements of skinfold thickness at the biceps, triceps, subscapular and suprailiac sites, and measurements of body density by underwater weighing were made on 260 male and 311 female subjects aged between 12 and 72 years. The younger subjects (up to age 15) were an approximately random sample of Glasgow school-children. All the other people were selected to include a range of different body builds in the various age-groupings.

Regression equations have been calculated for estimating body density from skinfold thickness at each of the four single sites and for all multiples of these sites, for both males and females in the separate age groups: 12–15 years, 16–19 years, 20–29 years, 30–39 years, 40–49 years, 50 years and over.

The standard error of the estimate of body density from skinfolds was reduced if multiple sites were used. Logarithmic transformation also slightly improved the relationships, but subtraction of a constant (1·8 mm), representing the thickness of a double layer of skin, had no influence. There was no significant difference between different groupings of multiple sites; for example the triceps, subscapular and supra-iliac was the best group but the value for it was not significantly different from that for the biceps, triceps and subscapular, nor from that for all four sites taken together.

The regression equations differ for the two sexes and alter with increasing age. With these equations, it is thus possible to calculate the total body fat in people of varying age from skinfold thickness measured at one or at several sites in the body.

A comparison of vitamin C status of elderly in–patients with that of elderly out–patients. By J. ANDREWS, *Department of Geriatric Medicine, West Middlesex Hospital, Isleworth*, SUSAN J. ATKINSON, B. D. RIDGE and CHRISTINE WYN-JONES, *Beecham Group Research Unit, Walton Oaks, Dorking Road, Tadworth, Surrey*

Kataria, Rao & Curtis (1965) first observed that the buffy layer vitamin C concentrations of elderly people living in institutions were lower than in those living at home. Platt, Eddy & Pellett (1963) had previously shown that, in general, smaller hospitals provided a better quality of food than larger hospitals.

During August 1972, when vitamin C-rich foods were relatively abundant, we measured the vitamin C concentrations in the blood of elderly women who were either patients in long-stay wards in two hospitals or attending as out-patients. The clinical diagnoses were essentially similar for the in-patients and the out-patients but the in-patients were the less mobile. The results are summarized in Table 1.

Table 1. *Buffy layer vitamin C concentrations of elderly women*

	No. of subjects	Age (mean and range) (years)	Buffy layer vitamin C mean $\pm$ SEM ($\mu g/10^8$ cells)
Out-patients	37	80 (68–90)	28·0 $\pm$ 1·8
Small hospital in-patients (19 beds)	16	82 (69–102)	20·0 $\pm$ 1·9
Large hospital in-patients (128 beds)	29	82 (67–98)	12·5 $\pm$ 0·8
All in-patients	45	82 (67–102)	15·2 $\pm$ 1·0

The out-patients had a significantly higher mean buffy layer vitamin C concentration than the in-patients in either hospital ($P < 0·001$). Also, the in-patients drawn from the small hospital had a significantly higher buffy layer vitamin C concentration than those from the large hospital ($P < 0·01$), both hospitals being in the same group.

The average age and the age-range were similar for the in-patients and out-patients. At the larger hospital, hot food was transferred to heated trolleys at about 11.20 hours for transport to the wards, where it was served about 20 min later. At the small hospital food was cooked next to the dining room and served immediately. Neither hospital served fresh fruit routinely and dietary information showed that the out-patients generally ate more fresh fruit than the in-patients.

It is well known that supplementation can raise vitamin concentrations in blood in the elderly (Andrews, Letcher & Brook, 1969) and in geriatric hospital patients (Brocklehurst, Griffiths, Taylor, Marks, Scott & Blackley, 1968). Our observations provide further supporting evidence that the low blood concentrations of vitamin C previously reported in elderly hospital patients are not a consequence of age or multiple pathology. They could be a direct consequence of reduced vitamin C intake associated with current large-scale catering practices.

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Biochemical characteristics of different forms of protein–energy malnutrition in rats. By C. R. C. HEARD, SYLVIA M. FRANGI and PAULINE M. WRIGHT, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

Alleyne & Scullard (1969) reported that the livers of malnourished children had low concentrations of glycogen and high activity of glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase: *EC* 3.1.3.9). These results were contrary to earlier reports, and we thought that they might reflect unknown differences in protein and energy intake. Experiments were carried out with groups of six young rats, initially 72 g, fed on measured or controlled quantities of diets of high- (HP) or low-protein (LP) value. Biochemical measurements, made in material obtained at *post-mortem*, after a 5 h fast, are shown in Table 1. Two other experiments gave consistently similar results.

Table 1. *Effect of protein and energy intake, on body-weight and some biochemical measurements in groups of six young rats fed for 4 weeks on the diets shown*

Diet Dietary regimen	HP *Ad lib.*	LP *Ad lib.*	HP–PF Pair-fed to LP	HP–fasted *Ad lib.;* 48 h fast
NDp:E ratio of diet	0·10	0·04	0·10	0·10
Body-weight (g)	216	81	112	189
Weight gain (g)	143***	9***	42	139–22
Food intake (g)	473***	274	269	—
Haemoglobin (g/l)	129***	101***	132	144**[a]
Plasma protein (g/l)	77***	56***	81	80
Blood glucose (g/l)	1·18*	0·96**	1·35***	0·62***
Plasma insulin (mU/l)	50**	23	19	24**
Ratio, insulin:glucose (μU/mg)[b]	39**	24	30	
Liver glycogen (mg/g)	56	70***	18***	4***
Liver glucose-6-phosphatase (U/g)	19·1***	11·6***	21·2***	17·3
Liver fat (mg/g dry wt)	103***	180***	98**	131*
Liver GOT[c] (U/g)	22·1***	38·8*	52·1	49·4**
Liver GPT[c] (U/g)	7·6***	1·7***	23·9*	15·6***
Muscle GOT (U/g)	51·6	45·2	42·1	37·9
Muscle GPT (U/g)	4·0***	1·5*	2·5	2·6*

HP, high-protein; LP, low-protein; NDp:E ratio, ratio of energy supplied by utilizable protein to total metabolizable energy.
Significance of differences between adjacent columns: *$P<0.05$; **$P<0.01$; ***$P<0.001$.
a, significant differences from HP rats.
b, calculated from mean values during 1 h oral glucose tolerance test.
c, GOT, GPT = aspartic and alanine aminotransferases respectively.

The HP group pair-fed to LP (HP–PF) group gave results which 'contradicted' those for the LP group, with respect to hepatic glycogen and glucose-6-phosphatase. Similar contrasts were found in alanine aminotransferase (*EC* 2.6.1.2) in liver and muscle, and they are compatible with gluconeogenesis being decreased in the LP group and increased in the HP–PF group. The effects of chronic undernutrition (HP–PF) may be compared with those of acute fasting (HP–fasted).

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Plasma amino acid concentrations and ratios in different forms of protein-energy malnutrition in rats. By P. G. Broadbent and C. R. C. Heard, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

Plasma amino acid concentrations were determined in young rats which, for 4 weeks, had consumed known amounts of diets of different protein content. Dietary treatment, food intakes and body-weights are reported in the previous communication (Heard, Frangi & Wright, 1973). Pooled samples of plasma from three rats in the

Table 1. *Mean plasma amino acid concentrations and ratios in protein-energy malnourished rats*

Diet	HP	LP	HP–PF	HP–fasted
No. of samples	3	3	3	2
No. of rats	9	9	9	6
	μmol/l	Concentration relative to HP value		
Taurine (N)	61	0·78	2·05	1·11
Aspartic	49	0·91	0·88	1·00
Threonine	279	0·16	0·74	0·84
Serine (N)	251	1·25	1·16	1·04
Glutamic	202	0·95	0·81	0·82
Glutamine (N)	677	0·60	0·78	0·80
Glycine (N)	151	1·52	1·74	2·12
Alanine	492	0·87	0·71	0·46
Citrulline	57	1·03	1·61	0·87
Cysteine	6	1·26	1·80	3·22
Valine (E)	150	0·53	0·79	1·00
Methionine (E)	103	0·34	0·33	0·38
Isoleucine (E)	80	0·45	0·90	1·10
Leucine (E)	126	0·59	0·95	1·11
Tyrosine	83	0·35	0·69	0·83
Phenylalanine	51	0·69	1·02	1·25
Asparagine	47	0·40	0·86	0·94
Ornithine	24	1·82	1·43	1·00
Histidine	60	1·05	0·78	1·06
Arginine	103	0·60	1·05	1·18
Total NH₂–N	3051	0·72	0·87	0·87
ΣN:ΣE	2·48	4·42	3·52	2·85

HP, high-protein; LP, low protein; HP–PF, HP group pair-fed to LP; N, non-essential; E, essential.

same group were analysed on a Locarte amino acid analyser. The mean results from two experiments are shown in Table 1. These for the rats given the low-protein diet (LP) resembled previous findings for protein-deficient dogs and malnourished children (Platt, 1968), and showed specific reduction in a number of essential amino acids. Methionine concentration was reduced in all the malnourished rats, and alanine was specifically reduced in the acutely fasted group (HP–fasted). The plasma amino acid ratio, calculated for four non-essential and four essential amino acids (Whitehead, 1964), was high in the LP rats.

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Variation in the intestinal dipeptidase activity during pregnancy and lactation in the rat. By B. A. ROLLS and J. W. G. PORTER, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

The food intake of the pregnant rat is slightly higher than that of the non-pregnant animal and that of the lactating animal is markedly higher (cf. Fell, Smith & Campbell, 1963; Crean & Rumsey, 1971), and it has been claimed that this increase is confined to the protein component of the diet when rats are allowed to select their own food (Leshner, Siegel & Collier, 1972). Although hypertrophy of the gut was observed by Fell *et al.* (1963) in the lactating rat, it is not clear whether the digestive and absorptive capacities of the small intestine are increased.

Dipeptidases, which are located largely within the wall of the small intestine, are important in the final stages of protein digestion and absorption. We have assayed the glycyl-L-leucine dipeptidase activity of extracts of the small intestine from groups of individual rats at different stages of pregnancy (two rats/group) and lactation (four rats/group), using the spectrophotometric method of Josefsson & Lindberg, 1965).

The glycyl-L-leucine dipeptidase activity of the intestinal mucosa increased slightly during pregnancy and strikingly during lactation over that found in non-pregnant controls. Mean values and standard deviations, relative to the control mean were:

	Mean	SD
Non-pregnant controls	1·000	0·055
End of 1st week of pregnancy	1·126	0·046NS
End of 2nd week of pregnancy	1·252	0·109NS
End of 3rd week of pregnancy	1·353	0·034**
End of 1st week of lactation	2·408	0·273**
End of 2nd week of lactation	3·080	0·546**
End of 3rd week of lactation	2·492	0·462**
End of 1st week after weaning	1·282	0·013*

Difference from control groups: NS, not significant; *, $P<0.05$; **, $P<0.01$.

The changes are broadly similar to those in the pattern of food intake during pregnancy and lactation reported by Crean & Rumsey (1971) and it seems possible that the increase in enzyme activity is a response to food intake. However, the peak of enzyme activity is at the 2nd week of lactation, whereas the peak of food consumption occurs during the 3rd week, and it is possible that some other stimulus, perhaps hormonal is responsible.

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Tentative estimation of the amount of amino acids absorbed during digestion of a protein–free diet. By A. RERAT, *I.N.R.A.–C.N.R.Z.*, 78350 *Jouy-en-Josas, France*

The quantitative study of the absorption of nutrients can be made by establishing,

at any moment after a meal, the arteriovenous differences in concentrations of the nutrients in the blood in the intestine, and by multiplying these differences by the corresponding blood flow-rate (Rerat, 1971*b*). With this object, various techniques have been devised: on the one hand, permanent cannulations of the intestinal portal vein and of the jugular vein (Arsac & Rerat, 1962) allowing spaced (Pion, Fauconneau & Rerat, 1964) or continuous (Aumaitre, Février, Rerat, Rigaud & Thivend, 1969) blood samplings; on the other hand, the chronic implantation of a flow probe connected with an electromagnetic Flowmeter Medicon K2000 allows measurement of the blood flow at any time in the portal vein (Rerat, 1971*a*). This
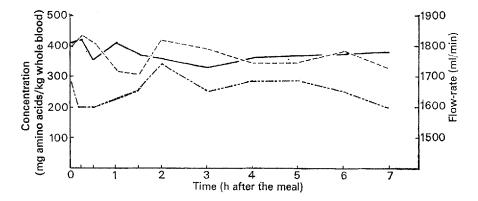

Fig. 1.   Concentration of amino acids in blood from the portal and jugular veins of pigs. ————, portal vein; — — —, jugular vein; --—--—--, flow-rate, portal vein.

combination of techniques previously applied to sugars (Aumaitre & Rerat, 1971) is now used to measure the quantitative absorption of amino acids, especially after the intake of a protein-free meal.

This method was applied to two Large White pigs, 40–45 kg live weight, receiving a protein-free diet (380 g maize starch, 30 g crude fibre), 15 h after a 'normal' meal (semi-synthetic diet with a balanced protein supply). The blood samples were taken at different time-intervals for 7 h after the meal and the free amino acids were determined in the samples (Pion & Rerat, 1967). Concerning the values obtained with the three protein-free diets offered to the animals, only the most characteristic ones are reported (Fig. 1 and Table 1).

During digestion, the concentration of free amino acids was generally higher in the jugular vein than in the portal vein. However, during certain periods (1–2 h and 4–6 h after the meal) this tendency was reversed, corresponding probably to the absorption of endogenous amino acids. In addition, it can be noted that the amount of total amino acids reabsorbed during the 7 h after the intake of a protein-free meal is rather large, but seems variable from one meal to another and according to the amino acid studied; it must be pointed out that the amount of reabsorbed lysine could exceed 1 g (Table 1).

Table 1.   *Amount of amino acids absorbed by pigs during 7 h after ingestion of a protein-free diet*

Expt no.	Mean portal blood flow* (ml/min)	Total amino acids (g)	Essential amino acids (g)	Lysine (g)	Methionine (g)
1	1650	16·6	4·26	1·15	0·56
2	1625	16·6	4·31	0·32	0·10
3	930	11·4	3·36	1·05	0·00

*During 8 h after the meal.

The values for amounts of amino acids recovered in the portal vein are in agreement with those found in the intestinal lumen during digestion of a protein-free diet (Rerat & Lougnon, 1963). It may be that the variations recorded are related to phenomena of destruction in the digestive tract or of metabolism in the gut wall, which would correspond to variations in the previous nutritional status of the pig. More experiments are necessary before more definite results can be given.

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Factors affecting free amino acid and urea nitrogen concentrations in the blood plasma of preruminant calves. By A. P. WILLIAMS and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Apart from the work of Leibholz (1965) on calves up to 4 weeks of age there is little published information on plasma amino acid (PAA) and plasma urea nitrogen (PUN) concentrations in preruminant calves. We are interested in the possibility of using such values to assess the efficiency of protein utilization and amino acid requirements in preruminant calves and to develop techniques which might be applied to the ruminating animal. To apply such techniques it is important to know how these values are influenced by factors such as animal-to-animal variation, age (Leibholz, 1965), circadian rhythms (Feigin, Beisel & Wannemacher, 1971), and time of feeding (Porter & Williams, 1963).

Friesian calves were fed on whole milk (approximately 0·05 kg/kg body-weight) at 10.00 and 17.00 hours. Samples of jugular blood were taken before feeding at 10.00 hours and at 1, 2, 3, 4, 5 and 6 h after feeding at 2, 3, 5, 7, 8 and 9 weeks of age. At 9 weeks samples were also taken at 8, 10, 12, 14, 16, 18, 20, 22 and 24 h after the 10.00 hours feed, and during these collections the calves were kept in darkness from 20.00 to 08.00 hours.

The mean values with standard errors at 5 weeks and 13.00 hours were 115 ± 8 mg PUN/l plasma (ten calves) and 1689 ± 95 μmol PAA/l (ten calves). In experiments with four calves, little variation was apparent in PUN values within individual calves at different ages between 2 and 9 weeks and the considerable animal-to-animal variations were shown consistently over this age-range. Neither total PAA concentrations nor those of individual amino acids showed any clear diurnal patterns over 24 h, but there was a small fall (approximately 10%) in PUN concentrations between 10.00 hours and 13.00 to 14.00 hours with recovery by 18.00 to 22.00 hours. These findings are in marked contrast to the considerable increases in PAA (Porter & Williams, 1963) and PUN concentrations (Eggum, 1970) after feeding observed in some other animals. This may be related to the slow release of protein from the milk clot which occurs in the abomasum of the preruminant calf.

Feeding with synthetic diets simulating milk, but deficient in tryptophan or methionine, led to markedly elevated PUN concentrations. Preliminary experiments in which these diets were supplemented with varying amounts of tryptophan or methionine led to depression of PUN concentration, indicating that this measurement might be of value in assessing the efficiency of protein utilization.

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Plasma concentrations of free amino acids in sheep in relation to time of feeding and protein intake. By J. L. MANGAN and P. C. WRIGHT, *ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

In monogastric animals plasma amino acid concentrations increase after a protein meal and the pattern is related to the protein composition (see Leathem, 1968, for review). With ruminants, Liebholz (1965) observed increases on feeding, but Purser, Klopfenstein & Cline (1966) found decreases with low-protein (80 g/kg) but increases with high-protein (125 g/kg) diets. Six adult Clun Forest sheep were given once daily 1000 g hay+200 g oats, and six Soay sheep half these amounts. After 3 weeks' conditioning, jugular blood was withdrawn before and 3 h after feeding. The mean concentrations (μmol/l) of individual amino acids in each group were as follows (Clun Forest, before/after feeding; Soay, before/after feeding):

urea (4770/5120; 5190/5280), glycine (548/385; 555/427),
glutamine (256/230; 227/246), valine (254/212; 198/170),
arginine (186/194; 180/127), alanine (181/154; 213/198),
citrulline (171/154; 133/96), lysine (157/122; 185/129),
leucine (124/96; 111/79), serine (118/95; 111/90),
ornithine (109/106; 114/87), taurine (98/66; 114/66),
threonine (100/79; 83/58), proline (93/85; 123/91),

isoleucine (93/71; 89/64), glutamic acid (79/64; 99/100),
histidine (69/65; 65/66); tyrosine (60/62; 62/42),
phenylalanine (54/44; 49/36), methionine (20/18; 22/25),
aspartic acid (12/19; 16/12), cystine (traces only).

The slight increases in urea, glutamine, aspartic acid, tyrosine and arginine with the Cluns, and in urea, glutamic acid, methionine, and histidine with the Soays were not significant. All other amino acids showed a decrease which was significant ($P<0.05$) for threonine, citrulline, glycine, alanine, valine, and isoleucine with the Cluns, and for taurine, threonine, citrulline, glycine, valine, isoleucine, leucine, lysine, and arginine with the Soays.

A Clun Forest and a Soay on the above diets were sampled at 09.00, 12.00, 15.00, 18.00, and 21.00 hours and the sampling continued for 60 h. Tryptophan, cystine, methionine, aspartic acid, carnosine, and 3-methyl-histidine (<35 μmol/l) did not vary greatly. Glutamic acid and glutamine (70–200 μmol/l; >200 μmol/l) increased after feeding. Glycine (300–700 μmol/l) and all the remaining plasma amino acids (35–170 μmol/l) decreased markedly for 6–9 h after feeding. This pattern was repeated the following day.

When the protein content of the diet was increased from 72 to 125 g/kg there were only slight increases in plasma amino acids and, on feeding, the concentrations decreased as before except for glutamine, urea, and ammonia.

Erythrocytes in both breeds of sheep have a high concentration of amino acids compared to plasma, particularly aspartic acid, threonine, glutamic acid, glycine, and ornithine. Glutamine and arginine were lower than in plasma. Equilibrium dialysis against Ringer–Locke solution showed that all the amino acids except citrulline would exchange from erythrocytes to plasma.

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Investigation of mechanisms operating in dye–binding procedures for the evaluation of protein quality. By A. L. LAKIN (introduced by F. AYLWARD), *Department of Food Science, University of Reading, Reading RG1 5AQ*

The use of dye-binding procedures for the evaluation of the nutritional quality of proteins is based on the high correlations which have been obtained between the amounts of dyes bound by proteins and their contents of basic amino acids (cf. Mossberg, 1970). The mechanisms which have been proposed for the dye–protein interaction (cf. Ashworth & Chaudry, 1962; Udy, 1971) may be summarized as follows: the dominant factor is an electrovalent association between dye anions and residues of basic amino acids, plus an additional amount of dye bound by hydrogen or hydrophobic bonding, or both; the extent of the secondary association being

determined by the molecular structures of the dye and the protein. This model has been investigated and has been found to be incomplete.

The dyes C.I. Acid Orange 10 ('Orange G'), C.I. Acid Orange 12 and C.I. Acid Black 1 ('Amido Black 10B') were purified by recrystallization, and their binding properties with different proteins were compared. The role of the structure of the dye molecule in secondary binding was confirmed because, for any given protein, the dyes were not bound in equivalent amounts. Similarly, the role of protein structure was confirmed because the ratio of the amounts of dyes bound was different for each protein.

When cellulose powder was substituted for the proteins, it was found that the amounts of dyes adsorbed were so small that hydrogen or hydrophobic bonding, or both, between these dyes and cellulose was negligible. It was postulated, therefore, that secondary binding would not occur with diethylaminoethyl cellulose and that this material should bind the dyes stoichiometrically. This was not so. The dyes were bound in excess of the determined anion exchange capacity and in different amounts: 111, 115 and 137% recovery of the basic groups being given by C.I. Acid Orange 10, C.I. Acid Orange 12, and C.I. Acid Black 1 respectively.

It was concluded that the overestimation of basic groups was caused by an association between dye anions from solution and dye anions bound by primary valency forces. This phenomenon, 'dye-to-dye association', must also occur when dye-binding procedures are used for the evaluation of protein quality and so it should be included in any model which is proposed for the mechanisms operating in these methods.

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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, Cambridge CB2 3DX*

It has been generally assumed that different methods for measuring the reactive lysine content of foods with either fluorodinitrobenzene (FDNB) or trinitrobenzene sulphonic acid (TNBS) give essentially the same results. However, Finot & Mauron (1972) found big differences when they applied these methods to α-N-formyl-(ε-N-deoxyfructosyl)-lysine (FFL), synthesized as a model of one of the Maillard compounds believed to be formed between glucose and the lysine units in proteins when the two are in contact under relatively mild conditions. They also found FFL to be unavailable as a source of lysine for rats.

We have confirmed the existence of these differences with FFL for three procedures (Table 1), though our absolute values differ to some extent from those of Booth (1971) and Finot & Mauron (1972). We have also applied these procedures to protein after contact with glucose at 37° for 30 d or at 121° for 15 min and to protein heated alone at a very high temperature (so that lysine units would be expected to form cross-linkages with other amino acids).

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

Material	Treatment	'Total' value after acid hydrolysis	FDNB value by difference (Roach, Sanderson & Williams, 1967)	FDNB value by direct method (Carpenter, 1960)	TNBS value by direct method (Kakade & Liener, 1969)
FFL*		53	53	3	80
'Ovalbumin 3, lactalbumin 2, glucose 5'					
(12% H_2O content)	30 d at 37°	60	54	24	67
	15 min at 121°	34	23	15	23
Bovine plasma albumin					
(13% H_2O content)	27 h at *c.* 150°	76	18	14	14

FDNB, fluorodinitrobenzene; TNBS, trinitrobenzene sulphonic acid; FFL, α-N-formyl-(ε-N-deoxyfructosyl)-lysine.

*Values are expressed as percentages of the theoretical lysine content of the molecule.

The mildly heated protein–glucose mixture has given results in line with those found for FFL and, as concluded by Finot & Mauron (1972), neither the FDNB 'difference' method nor the TNBS procedure appears able to measure the full extent of the lysine binding that occurs under these conditions. With materials heated at high temperature, whether or not sugar is present, the results by the different procedures are in better agreement.

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