was also much higher in the older animals. Thus the reduction in the efficiency of utilization of dietary N from 33 to 20%, as live weight increased from 150 to 240 kg, is accounted for by an increase in urinary N. It would appear that with rapidly growing cattle there is an increase in endogenous urinary N with increasing level of performance i.e. live-weight gain, and with increasing stage of maturity.

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The One Hundred and Sixty-fifth Meeting of The Nutrition Society was held at Queen Elizabeth College, London, W 8 on Friday, 22 May 1964, at 10.15 am, when the following papers were read:

Further studies on copper sulphate and molasses distillers dried solubles as growth stimulants in the diet of growing pigs. By R. S. Barber, R. Braude and K. G. Mitchell, National Institute for Research in Dairying, Shinfield, Reading

In previous work (Barber, Braude & Mitchell, 1962), the addition of  $2\frac{1}{2}\%$  molasses distillers dried solubles (E. C. Feed; National Chemical Products Ltd) to a diet containing animal protein significantly improved growth rate in growing pigs by  $5\cdot1\%$ , whereas addition of copper sulphate (supplying 250 p.p.m. Cu) increased growth rate by  $14\cdot5\%$ . Although both additives similarly increased rate of food consumption, only copper improved the efficiency of food utilization.

The present experiment was carried out to obtain further information on E. C. Feed and to investigate whether there was any supplementary effect on performance when both copper and E. C. Feed were added to the diet as indicated in a preliminary report by Robinson, Coey, Holme & Kormos (1959–60). Forty-eight individually fed Large White pigs, in twelve lots of four litter-mates each and on experiment from 9 weeks of age to bacon weight, were used to compare the effect on performance and carcass quality of the addition to the diet either of copper sulphate (250 p.p.m. Cu), or  $2\frac{1}{2}\%$  E. C. Feed or of both additives together. The basal diet fed and the method of feeding were similar to that used previously (Barber et al. 1962) except that a lower level of white-fish meal was included (7% reduced to 3% from 120 lb live weight), and the pigs were restricted to a daily maximum of 6 lb meal instead of  $6\frac{1}{2}$  lb.

Copper sulphate significantly improved growth rate (by 8.0%) and efficiency of food utilization (by 4.6%) whereas E. C. Feed had no significant effect on either. Pigs given both copper and E. C. Feed performed similarly to those given copper alone. There were no significant differences between any of the treatments in rate of food consumption; dressing percentage; carcass length; shoulder depth; shoulder

and loin backfat thickness; eye muscle width, depth of colour; fat thickness round the eye muscle or back rasher score.

We wish to thank Mr J. A. Wakelam, National Chemical Products Ltd for supplying the E. C. Feed.

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The effects of a single large oral dose of vitamin A or carotene on vitamin A in the liver of the goat. By Edwin C. Owen and R. Proudfoot, Biochemistry Department, Hannah Dairy Research Institute, Ayr

Vitamin A itself is so readily absorbed that in rat and man toxicity results with large doses. If all the carotene (2–4 g/day) ingested by a grazing cow were changed to vitamin A and absorbed, vitamin A toxicity would probably result, as suggested by Owen (1964). The comparative absorbabilities of  $\beta$ -carotene and vitamin A were therefore directly compared in the goat with results (Table 1) which show that (assuming equal initial hepatic concentrations)  $\beta$ -carotene caused a 38% and vitamin A an 800% increase of hepatic vitamin A.

Table 1. Effects of oral vitamin A or  $\beta$ -carotene on tissue concentrations of vitamin A in the goat

Goat		Vitamin A (i.u./g tissue)			
no.	Treatment	Liver	Kidney	Brain	
I	None	159.7	${5.5 \atop 5.6}$ ${5.6}$	4.8	
2	4.5 g synthetic vitamin A in groundnut oil	1259.2	$5.6 \atop 5.8 \atop 5.7$	3.5	
3	5 g $\beta$ -carotene in groundnut oil	217.4	$\binom{6.0}{6.5}$ $6.3$	3.6	

#### REFERENCE

Owen, E. C. (1964). Wld Rev. Nutr Diet. (In the Press.)

Sex differences in the lipid response to dietary carbohydrate. By I. Macdonald and A. Nowakowska, Department of Physiology, Guy's Hospital Medical School, London, SE 1

It has been shown that differences exist in the lipid response of the serum and adipose tissue of adult men to diets containing large quantities of either starch or sucrose (Macdonald & Braithwaite, 1964). The differences were in the total fasting serum lipid level, which was raised on the sucrose diet (mainly as glycerides) and in

the fatty acid pattern in the serum and adipose tissue, which did not change in the same way in response to each carbohydrate.

As the lipid response of the adult men to sucrose was similar to that seen in ischaemic heart disease and as adult women have a reduced incidence of this condition until the menopause, it was felt that it would be of interest to study the effect of comparable diets in adult, pre-menopausal females.

Five healthy female volunteers aged 21–25 years were given a diet comparable to that previously given to the males. In brief this consisted of 500 g daily of maize starch or of sucrose for 25 days with ad lib. quantities of lean meat and green vegetables. Blood and adipose tissue samples were taken after 4, 11, 18 and 25 days on the diet. The serum lipid was fractionated on silicic acid columns and the fatty acid patterns of the serum and adipose tissue were determined by gas chromatography.

It was found that the serum fractions showed the same response to the starch diet in both men and women. The response to the sucrose diet was, however, different as is seen in the table.

Changes in the serum lipid fractions associated with a high-sucrose diet for 25 days in men and women

	Total lipid	Sterol esters	Glycerides	Phospholipid	Cholesterol
Males	-+-	0	+	0	+
Females	_	0		_	-
	+ =Increase - =Decrease $\bigcirc =$ Unaltered $Compa$		ared with contr	rol (P<0.025)	

The fatty acid in the serum showed the same changes in both sexes on the sucrose diet but on the starch diet the fatty acids of the serum did not respond in a similar manner in each sex. The fatty acid of the adipose tissue did not change markedly in the females on either dietary carbohydrate diet, in contrast to the males.

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# Retinal degeneration and other signs of vitamin A deficiency in the cat.

By Patricia P. Scott and J. P. Greaves,\* Physiology Department, Royal Free Hospital School of Medicine, London, WC 1

Kittens were reared on a semi-purified diet consisting of casein 35%; sucrose or dextrin 36.7%; lard 10%; arachis oil 12%; sugar-beet residue 3%; salt mixture 2% and vitamins. They grew normally until they were about 4 months old. Subsequently growth rate declined, final weights being 15-40% below that of adults on other adequate diets. At termination, fat stores were within normal limits, but liver, kidney and skeletal muscle weights were subnormal, both for the animal's age and for its body-weight.

<sup>\*</sup>Present address: Ministry of Agriculture, Fisheries and Food, Horseferry Road, London, SW1.

Some of the cats received about 250 i.u. vitamin A/day in their food, the remainder were supplemented with vitamin A palmitate and received more than 2000 i.u./day. Both groups showed signs of vitamin A deficiency; the signs developed more rapidly on the sucrose—casein diet than on dextrin—casein, and on the low vitamin A intake than the high.

After 6–20 months, according to the diet, the pupils were observed to be dilated under normal lighting. A severe but transient photophobia was the earliest sign found. The pupillary reflex to light became more and more delayed, while the retinal vessels became extremely attenuated. At this stage the cat was unable to avoid objects in its path or to jump off a low stool. At termination, progressive retinal degeneration was apparent histologically, beginning with the visual elements, followed by the outer nuclear and plexiform layers. The inner nuclear layer was affected in the longest experiments but the ganglion cells remained normal. As the syndrome developed, keratitis and corneal vascularization appeared; typical changes of squamous metaplasia were also apparent in the mouth and skin while the fur was loose and unkempt. These latter changes were observed in the vitamin A-deficient cat by Gershoff, Andron, Hegsted & Lentini (1957), but they did not refer to retinal degeneration. Reproduction was defective, foetuses died in utero or were aborted.

Cats on a casein-based diet were apparently unable to store or utilize vitamin A normally. This was apparent in the liver and plasma and especially in the kidneys all of which had low concentrations at post-mortem. But very low vitamin A levels can be achieved in these organs on a meat diet without signs of deficiency (Moore, Sharman & Scott, 1963); thus casein may be an inadequate protein for cats.

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Variations in the distribution of calcium in the femure of growing and adult cats on high and low calcium intakes. By E. R. Humphreys and Patricia P. Scott, *Physiology Department*, *Royal Free Hospital School of Medicine*, London, WC 1

The aim of the study was to devise a method for determining the state of mineralization of the femurs of cats. Considerable variation in total femur calcium was apparent in growing animals which were maintained on similar dietary régimes, but reductions of femur Ca which occurred as a result of a low-Ca diet, took place preferentially from the ends of the femurs rather than from the shafts. At the same time there was an apparent reduction in overall mineralization expressed as a fraction of the fat-free bone weight.

End and shaft analyses of growing male and female kittens up to 6 months of age on low- or high-Ca diets showed that the ratio shaft Ca: ends Ca increased as a result of low dietary Ca (see table).

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Calcium in the femurs of normal and calcium-deficient cats expressed as a percentage of fat-free weight of bone

Kittens	Normal (21)	Ca-deficient (18)	P
Shaft Ends Shaft Ca Ends Ca	21·7±0·24 15·2±0·48 0·66±0·014	19·2 ±0·49 10·8 ±0·36 0·81 ±0·022	<0.001 <0.001 <0.001
Adults Shaft Ends Shaft Ca Ends Ca	(7) 25·8±0·97 20·8±0·59 0·75±0·035	(7) 25·1±0·40 20·5±0·94 0·71±0·018	Not significant Not significant Not significant

Thus a high shaft: ends Ca ratio is indicative of Ca deprivation in the kitten.

Results for male and female adults showed no significant differences between normal and Ca-deprived cats; Ca deprivation, in these cases, was achieved by lactation on a low Ca intake.

No significant differences were detectable between the S: E ratios for male and female kittens and adults.

# Variations in serum protein levels associated with a high carbohydrate intake in man. By Betty L. Coles and I. Macdonald, Departments of Physiology, Royal Free Hospital and Guy's Hospital Medical Schools, London

Previous experiments on the effect of the addition of carbohydrate to low-protein diets in animals showed that such additions generally exaggerate the fall in serum albumen (Coles & Macdonald, 1963), an effect which would seem to be the reverse of protein sparing.

In view of this the opportunity was taken of studying the serum proteins in seven adult males on a high-carbohydrate: low-fat diet and whose protein intakes were at a level generally considered to be adequate (Macdonald & Braithwaite, 1964). Two dietary carbohydrates were consumed namely maize starch and sucrose and the experimental period lasted 25 days on each carbohydrate. The mean protein intake of each subject is seen in the table.

The serum proteins were separated by paper electrophoresis and the individual protein fractions estimated by the method of Levin & Oberholzer (1953). The total serum protein was estimated with a micro-Kjeldahl technique.

The mean daily protein intake (g/kg body-weight) of each subject on the two dietary carbohydrate régimes

Subject	N.G.	J.J.	I.M.	V.M.	G.P.	C.R.	P.S.
Starch diet	0.83	1.09	<b>o</b> ·96	1.55	0.90	0.70	1.04
Sucrose diet	1.30	1.46	1.40	1.10	1.24	0.67	1.04

The results showed that the total serum protein level fell significantly mainly due to the albumen. The  $\gamma$ -globulins rose significantly. Apart from a fall in the  $\beta$ -globulins in the sucrose-eaters which was not seen in the starch-eaters, the serum protein findings were similar in the two dietary carbohydrate régimes.

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The determination of gross energy values in nutritional studies using a conventional and ballistic bomb calorimeter. By D. W. Robinson, University of Liverpool, Veterinary Field Station, Neston, Cheshire and D. Cole, M. H. Clarke and H. S. Bayley, University of London, Wye College, Ashford, Kent

Current experimental work on the dietary energy component has afforded the opportunity to make a comparison between the rapid determination of gross energy values of food and faeces using a ballistic bomb calorimeter and those determined on the more conventional static bomb calorimeter. The summarized data from over 600 determinations are given in the table to illustrate the repeatability and relative accuracy of the two methods.

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% of determinations with
                                         each accuracy range
                                             2%
% accuracy between determinations
                                                   2\frac{1}{2}\%
                                           50.0
Conventional calorimeter—duplicates 16.7
                                                   100.0
Ballistic calorimeter
                       —duplicates 34.0
                                            63.0
                                                    73.0
                                                           78·1
                       -triplicates
                                      82.0 92.0
                                                    96.1
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When duplicates of each sample are taken for analysis 50% of the duplicate determinations are accurate to within 2% of each other and all are within  $2\frac{1}{2}\%$  when the conventional bomb calorimeter is used; 63% of duplicate determinations on the ballistic bomb calorimeter are accurate to within 2% and 73% to within  $2\frac{1}{2}\%$ . With a further single determination (i.e. triplicate determinations) a 2% accuracy can be obtained with 92% of all samples examined. The range of variability obtained with the ballistic bomb calorimeter is only slightly greater than that obtained with the conventional calorimeter, and against this must be offset the time factor since it has proved possible to complete twelve determinations/h with the ballistic bomb calorimeter compared with one/h on the conventional bomb calorimeter. Using the ballistic bomb calorimeter the above patterns of repeatability were virtually the same in data from three separate trials with different operators and with samples from pig and poultry trials. Our findings with regard to the preparation of materials are in agreement with those of Miller & Payne (1959) and Sibbald (1963) that determinations on finely powdered preparations are far more accurate than when pelleted.

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The effect of the inhibition of egg laying on the utilization of dietary energy in the domestic fowl. By H. S. Bayley, M. H. Clarke, P. Hunton and A. H. Sykes, Wye College (University of London), Ashford, Kent

Egg laying can be inhibited by the oral administration of  $\tau$ -( $\alpha$ -methyl-allyl)-6-methyl dithiobiurea included at low levels in the ration. This effect of the drug provides an opportunity to study the utilization of energy in laying and non-laying birds. Comparisons of the values obtained could give an indication of the partition of energy between maintenance and egg production, provided that there was no change in carcass composition.

Twenty Light Sussex pullets were given a diet containing the drug at a level of 0.02% from the age of 21 weeks. No eggs were produced by the birds which otherwise appeared sexually mature. Food intakes were recorded daily from the age of 28 weeks and were stable over the next 4 weeks. At 32 weeks of age five birds were killed and the drug was removed from the diets of ten others. Five of these were killed at an age of 34 weeks and the other five at 36 weeks. The control group of five birds continued to receive the drug and were killed at 36 weeks of age. The dry matter, ether-soluble material, and nitrogen contents of all the carcasses were determined. Food intakes and faeces outputs for each bird were recorded daily and determinations of gross energy values of food and faeces allowed the calculation of metabolizable energy intakes.

The first egg was laid 9 days, on average, after the drug was removed from the diet, and the food intake increased from 54 to 117 g/day over 18 days. Most of this increase took place in the first 3 days. Birds killed at 34 weeks contained significantly more fat than those killed at 32 weeks, but there was no difference between those killed at 34 and 36 weeks. There was slightly less fat in the control birds killed at 36 weeks than in the birds killed at 32 weeks but this difference was not significant. There were no differences in the nitrogen contents of any of the groups of birds and the differences in dry-matter contents were directly related to differences in fat content.

The results indicate that the increased dietary energy intake was used initially for body fat synthesis as well as for yolk production. Once egg laying began the increased energy was used mainly for egg production since from this stage there was no significant increase in the amount of fat in the carcasses. The drug appears to have a twofold effect at the level employed; reducing appetite and inhibiting the development of follicles. The changes in the composition of the carcasses associated with the removal of the drug preclude the ready partition of the dietary energy between maintenance and egg production.

# Some differences in the composition of broiler and free range chickens.

By Margaret S. Vipond, Jean Robertson and D. Tapsfield, Ministry of Agriculture, Fisheries and Food, London, SW 1

Estimates of food consumption in the United Kingdom show that the consumption of poultry rose from a prewar figure of 5·1 lb/head year to 14·6 lb in 1962. The United Kingdom Agricultural Statistics for June 1961 showed that 85% of the table

birds produced were broiler chickens, normally slaughtered at a live weight of  $3\frac{1}{2}$  lb (Ministry of Agriculture, Fisheries and Food; Department of Agriculture and Fisheries for Scotland; Ministry of Agriculture, Northern Ireland, 1964).

To investigate the possibility of differences in the composition and distribution of the carcass meat between broilers (B) and free range birds (FR), analyses of four types of birds were made. Different feeding regimens were used in the production of the two breeds of free range chickens.

Table 1

$\mathbf{Breed}$	Type of bird	No. of birds	Age (weeks)	Initial dressed wt* (lb)
Chunky 706	Fresh B <sub>1</sub>	12	9	2.7
Chunky 706	Frozen B <sub>2</sub>	12	9	2.7
Sykes hybrid 3	Fresh FR <sub>1</sub>	12	20	3.4
Light Sussex × Rhode Island Red	Fresh FR <sub>2</sub>	12	18	3.8

<sup>\*</sup>Does not include the giblets.

Half the birds of each breed and type were dissected and analysed raw; the remainder were roasted under identical conditions at 350°F without additional fat, before analysis. Duplicate samples of the breast muscles (pectoralis major and minor) and the flesh from the leg, wing and back (red meat) were analysed for thiamine, fat and protein; the following statistically significant results (P < 0.01) were found in cooked birds:

- (1) The edible portion (which includes the skin) expressed as a percentage of the initial dressed weight was higher in the fresh broilers (42.8%) and free range birds (43.8, 45.5%) than in the frozen broilers (38.9%). The breast meat of FR<sub>1</sub> made up 35% of the edible portion, 4% more than in the other birds.
- (2) Both the breast and red meat of groups FR<sub>1</sub> and FR<sub>2</sub> contained more thiamine than the broilers; both types of meat in FR<sub>1</sub> also contained less fat than the broiler meat.

Table 2. Chemical analyses of cooked birds\*

	Breast	t meat	Red meat		
Type of bird	Fat (%)	Thiamine (µg/100 g)	Fat (%)	Thiamine (µg/100 g)	
Fresh B <sub>1</sub> Frozen B <sub>2</sub> Fresh FR <sub>1</sub>	2·84±0·40 2·49±0·17 1·13±0·13	25·2±1·15 21·9±2·11 42·6±2·31	8·15±0·70 8·35±0·49 4·13±0·28	$28.8 \pm 3.12$ $26.7 \pm 1.03$ $52.7 \pm 4.92$	
Fresh FR <sub>2</sub>	2·55±0·37	32·6 ± 1·46	$8.31 \pm 0.68$	34·8±1·50	

<sup>\*</sup>Mean values with their standard errors.

(3) The red meat of FR<sub>1</sub> contained 29.5% protein  $(N \times 6.25)$ ; this was 2% more than the corresponding broiler meat.

The full results will be published elsewhere.

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Hydroxylysine as a stimulant in microbiological assays for lysine. By K. J. Carpenter, School of Agriculture, University of Cambridge and A. W. Hartley and L. D. Ward, Spillers Ltd Central Laboratory, Cambridge

Sauberlich (1957) in a preliminary communication reported without experimental detail that the presence of  $\delta$ -hydroxylysine (HOL) in samples assayed for lysine with Leuconostoc mesenteroides P-60 caused considerable error due to the synergistic effect it had with lysine, and that an improved response curve was obtained by the presence of a constant high level of HOL. However, others (Ågren, 1951; Pomeranz & Miller, 1963) have only found small effects from HOL, and the assay medium (Steele, Sauberlich, Reynolds & Baumann, 1949) sold in dehydrated form for use with this assay still contains no HOL (Difco Laboratories, 1960).

Our findings, in both laboratories, are in entire agreement with those of Sauberlich (1957). Typical results with a rehydrated 'Difco' medium, the tubes being autoclaved before inoculation and then incubated for 72 h at 37° (Steele et al. 1949) were:

Similar responses to HOL in the presence of low levels of lysine were obtained with freshly prepared medium steamed lightly before inoculation, and also when growth was measured turbidimetrically after 24 h at 34° following Schiaffino, McGuire & Loy (1958).

Two samples of HOL showed similar activity; one had been prepared by Dr Partridge from hydrolysed gelatin, the other was supplied commercially from the same source (L. Light & Co. Ltd, Colnbrook, Bucks) as the HOL reported by Pomeranz & Miller (1963) to have negligible effect. Each sample was resolved into two peaks on the Technicon amino acid analyser (Technicon Ltd, Chertsey, Surrey), the extra peak presumably being D-allohydroxylysine formed by partial epimerization of L-hydroxylysine at the  $\alpha$ -carbon during acid hydrolysis (Greenstein & Winitz, 1961). The isomers have not been tested separately.

Discrepancies between laboratories may be due to differences in media, organism etc., but the phenomenon has been encountered in at least one other laboratory (S. A. Price, private communication) and it seems essential to take precautions. We have chosen a standard 50  $\mu$ g HOL addition/tube, and results then approach those obtained by column chromatography (Technicon procedure):

	Lysine (g/16 g N)						
	Hydroxylysine	<u> </u>					
	(g/16 g N)		(Leuconostoc; Difco				
Test material	(Column)	(Leuconostoc; Difco)	$+$ 50 $\mu \mathrm{g}$ HOL)	(Column)			
Cod fillets, No. 34	0.022	9.7	9.5	9.2			
Fish flour, X.385	0.31	10.4	8.8	7 <b>.7</b>			
Meat meal, MM 18	o·33	10.0	7.0	6.7			

The explanation for the remaining discrepancies is still being sought; betweenassay variability with the microbiological assay has been unsatisfactory. No synergistic effect of HOL has been found for the growth response of chicks to lysine (Carpenter & Milner, unpublished).

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Carbohydrate preference in the rat. By R. H. J. Watson, Department of Nutrition, Queen Elizabeth College, University of London, Campden Hill, London, W 8

There is now considerable evidence that it is no longer tenable to regard different carbohydrates as nutritionally equivalent. For example, Macdonald & Braithwaite (1964) showed that starch and sucrose produced different patterns of lipids in the serum and depot fat of their subjects. Sognnaes (1948) showed that rats fed dextrin during pregnancy and lactation produced offspring with less dental caries than those fed sucrose. We know that in man there are marked individual preferences for different types of carbohydrate, but it is not known to what extent such preferences are due to innate differences in biochemical constitution and hence in nutritional needs, or are due to acquired habits based on earlier nutritional experience.

An experiment using rats was undertaken in which it was possible to control the early nutritional experience, and thus discover the preferences for different types of carbohydrate which may reflect differences in innate needs. During the critical phase of the experiment, which began when the animals were 100 days old, they were required to choose from diets equal in caloric density, but in which the carbohydrate portion was either glucose, sucrose, or dextrin (from maize starch). From this phase of the experiment (24 days) it was clear that there were marked differences between individual animals, and a significant difference between the sexes. Males preferred a relatively higher proportion of dextrin in their diets, whereas females preferred a relatively higher proportion of sucrose.

During the next phase of the experiment (24 days) the animals were able to select a diet in which they could vary the proportions of protein (casein), fat (arachis oil), and carbohydrate to their own liking. The carbohydrate for each animal was a mixture of glucose, sucrose, and dextrin based on its own preferences as shown in the previous phase of the experiment. Again marked individual differences occurred. During the last phase of the experiment (8 days) the animals' performance on a test of exploratory behaviour was investigated, and finally carcass analyses were undertaken.

A number of correlations have been discovered between the preferences and the physiological and behavioural measures. The results suggest factors which might influence carbohydrate preferences,

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Food habits of West Indian immigrants. By J. C. McKenzie and Pamela Mumford, Department of Nutrition, Queen Elizabeth College, University of London, Campden Hill, London, W 8

In recent years a number of studies have been made of the immigrant, though no extensive investigation appears to have been undertaken into their food habits. The present investigation is designed firstly to test the frequently expressed belief that the food habits of immigrant groups are slow to change to those of the country which they enter and secondly to try to indicate the pressures making for such changes as do occur.

This interim paper presents information gained from interviews designed to pre-test our survey techniques. The sample consisted of thirty-nine W. Indian housewives: thirteen attending a hospital prenatal clinic and twenty-five living in Notting Hill Gate.

The results in Tables 1 and 2 indicate that these families maintained a strong interest in W. Indian foods after their arrival in this country.

Table 1. 'As I read you a list of various kinds of foods, would you tell me if you have any of them regularly—say at least once a fortnight?'

(% of housewives regularly using each food)

Salt fish	61	Stewed fruit and custard	34
Fish and chips	53	Yam	79
Fresh apple	100	Green bananas	84
Salted pigs tail	34	Eggs and bacon	95
Frozen peas	32	Rice and Peas	97
Stewed beef flank	92	Sausages	68

Average % eating W. Indian foods regularly: 75 Average % eating other foods regularly: 68 W. Indian food dishes in bold-face type.

Table 2. 'I have another list of foods here—would you tell me if you often buy any of them?'

(% of housewives often buying each food)

Tinned meat	42	Fresh milk	100
Butter	100	Eggs	100
Lard	47	Creamed coconut	37
Red peas	76	Margarine	87
Heavy W. Indian bread	53	Corn meal	92
Gungo peas	82	Hot pepper pickles	71
White flour	97	Sweetened condensed milk	71
Cooking oil	87	Cheese	95

Average % purchasing W. Indian foods: 71 Average % purchasing other foods: 84 W. Indian dishes in bold-face type. Most families quickly adopted some English foods, but these were mostly snacks or subsidiary meals such as breakfast. Of the main meals eaten at home on the previous day, 85% had included W. Indian foods and dishes. The adherence to W. Indian foods was true even amongst those who had lived for more than 5 years in Britain.

# Overeating low-protein diets by adult man. By D. S. MILLER and PAMELA MUMFORD, Queen Elizabeth College, University of London, London, W 8

The work of Miller & Payne (1962) showed that young growing animals (pigs and rats) could maintain weight on a wide range of caloric intakes if the concentration of protein was varied. The present work was undertaken to see whether adult human beings can also consume 'excess' calories without putting on weight. For this purpose, diets containing about 2% of protein calories were constructed from ordinary foods. These were acceptable for long periods in the required amounts. Calories derived from fat and carbohydrates were equally divided.

The authors measured their customary food intake for 7 weeks and found their weekly average intake of calories (D.S.M. 2500 kcal; P.M. 2400 kcal), and protein (D.S.M. 9%, P.M. 12% of the calories) to be constant. During a second period of 6 weeks a regimen high in calories (D.S.M. 3400 kcal; P.M. 2900 kcal) and low in protein (D.S.M. 2.4%, P.M. 2.5% of the calories) was consumed. Finally there was another free-choice period during which the caloric intake returned to normal. Throughout, body-weight remained substantially constant. No differences were found in activity as measured by pedometer. Urinary output of nitrogen, sulphur and creatinine was determined during each period; basal metabolic rate and total body potassium were also measured.

The 'excess' caloric intakes of the second over the first period were 39 000 kcal for D.S.M. and 23 500 kcal for P.M., corresponding to 4:3 kg and 2:6 kg of fat respectively, amounts which could easily have been detected as changes in bodyweight. The possible gain in fat could have been offset by losses of lean body mass (estimated loss of 0.5 g K/day; see Christian, Combs & Kessler, 1963), or specifically of protein (estimated loss of 2-3 g N/day), but during the final period when the N and K losses were rapidly recouped the expected increase of body-weight was not observed. Thus either fat gain was exactly compensated by a loss of water in both subjects (D.S.M. 1 gal; P.M. 0.25 gal), or the excess food was katabolized and the energy dissipated as heat (D.S.M. 44 W; P.M. 24 W). In view of the earlier work with pigs the latter explanation is preferred. The results are consistent with work of Ashworth, Creedy, Hunt, Mahon & Newland (1962).

We are indebted to Mr Godfrey of the M.R.C. Radiological Protection Service, Sutton, for the determination of body K.

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Urinary sulphur as a measure of the protein value of diets. By D. S. MILLER and Pamela Mumford, Queen Elizabeth College, University of London, London, W 8

Miller & Donoso (1963) have shown that the protein value of diets from many parts of the world is limited by their content of the sulphur amino acids, and have proposed a method for 'scoring' diets based on their sulphur: nitrogen ratios. Since urinary S and N reflect the intake of these elements it has been suggested that these parameters could be used as indices of the quantity and quality of protein consumed ((USA) National Research Council, 1963), and that the S: creatinine ratio of casual urine samples might be sufficient to characterize the protein value of the diet. Difficulties have been found in the application of the method and a number of refinements are proposed.

Urine collections over 24 h rather than casual samples are necessary, since post-prandial excretion of N and S rises to a maximum, whereas creatinine excretion is independent of food intake. This removes the necessity for the proposed determination of creatinine as an index of daily urine production, and we have measured simply the S excretion/day, thus avoiding variations in creatinine excretion due to differences in body-weight. Finally, we have taken urinary N minus endogenous N (146 kg<sup>0.73</sup> body-weight; Brody, 1945) as the index of the quantity of protein consumed.

Urine samples were collected from volunteers eating their customary diets, or diets especially constructed to provide low protein, high protein and low 'score'. A good correlation was found between protein intake and urinary N minus endogenous N, provided that diets differing widely from the customary diet were consumed for a sufficient time to achieve steady values. There was also a good correlation between the quantity of net dietary-protein (equivalent to Reference Protein; FAO, 1957) and the urinary sulphate S (13 mg sulphate S/g net dietary-protein): endogenous sulphate S was negligible.

The relationships have been investigated only with adults and the dietary data have been calculated on the assumption that there was an adequate intake of calories for protein synthesis. If the method is found applicable to all physiological groups, it has much to recommend it for dietary surveys, especially for assessing the distribution of protein foods within the family: the adequacy of the diet with respect to calories might be judged by the level of ketone bodies in the urine.

We are indebted to Miss Susan Bickerstaff for technical assistance.

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Nutritional factors involved in the production of atherosclerosis in rabbits by semi-synthetic diets of low cholesterol content. By A. N. Howard, G. A. Gresham, D. Jones and I. W. Jennings, *Department of Pathology*, *University of Cambridge* 

Rabbits given a semi-synthetic diet of low cholesterol content develop hyper-cholesterolaemia and sudanophilic lesions in the aorta and coronary arteries (Gresham & Howard, 1962). Earlier work by Malmros & Wigand (1959), suggested that the disease could be attributed to a deficiency of essential fatty acids. This was not confirmed in our own experiments because the addition of maize oil did not prevent the atherosclerosis and hypercholesterolaemia, although they were much reduced.

As the semi-synthetic diet contained all known essential nutrients the disease could be caused either by a deficiency of an unknown nutritional factor or by the presence of a toxic agent in the diet. In order to investigate further the nutritional factors involved, groups of rabbits were fed on a basic atherogenic diet containing beef tallow 20%, casein 25%, vitamin mixture 1.2%, salt mixture 4%, cellophane 15%, maize starch 20%, sucrose 7%, glucose 5%, potassium acetate 2.5%; variations of this diet were made by replacing various constituents by other 'natural' products. Animals were given the diets for 16 weeks and the aortas were examined macroscopically for sudanophilic lesions in the gross after staining by the method of Holman, McGill, Strong & Geer (1958).

Replacement of the beef fat by maize oil, vitamin mix by yeast and cabbage, maize starch by maize meal, mineral mix by bone meal, and casein by purified soya-bean protein did not prevent the aortic atherosclerosis or hypercholesterolaemia. However, replacement of casein by hexane-extracted soya-bean meal was effective in preventing the arterial lesions and reducing the hypercholesterolaemia. This result suggests that the diet necessary to maintain a healthy aorta in the rabbit contains a nutrient which is present in hexane-extracted soya-bean meal. It is unlikely that a toxic factor was present since no one constituent was essential for the production of the disease. Also rabbits given a mixture of a 'natural' diet and the basic atherogenic diet did not have the disease. The possible nature of this nutritional factor is discussed.

This work was supported by Public Health Service Grant H-6300, National Institutes of Health, Bethesda, Md, USA, and a grant to one of us (D.J.) from the H. E. Durham Fund, King's College, Cambridge.

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Gresham, G. A. & Howard, A. N. (1962). Arch. Path. 74, 1. Holman, R. L., McGill, H. C., Strong, J. P. & Geer, J. C. (1958). Lab. Invest. 7, 4. Malmros, H. J. & Wigand, G. (1959). Lancet, ii, 749. The influence of urea and other dietary supplements on the nitrogen content of the digesta passing to the duodenum of hay-fed sheep. By R. N. B. Kay and A. T. Phillipson,\* Rowett Research Institute, Bucksburn, Aberdeen

The three sheep used in these experiments had re-entrant duodenal cannulas placed 5–8 cm beyond the pylorus. These formed an external loop through which all the digesta entering the duodenum passed. For 24 h periods the cannulas were disconnected and the flow of digesta from the proximal cannula was collected, 100 ml at a time, sampled and returned to the distal cannula, its volume restored by addition of digesta collected previously. Paper impregnated with chromium sesquioxide was introduced into the rumen with each meal and the recovery of the chromium in the 24 h digesta flow indicated the divergence of the recorded flow from the average normal flow. The amount recovered was usually less than that administered, suggesting that the collection procedure depressed duodenal flow to some extent.

The sheep received 700 g of rather poor hay, divided into two meals. This was supplemented with 75 g of flaked maize and with infusions of urea and of fatty acids given continuously into the rumen. The hay alone supplied about 6–7 g of total nitrogen/24 h but the amounts of N flowing to the duodenum, adjusted to the 'normal' flow by use of the chromium recovery, were 2–3 g greater than this. This reflects the addition of considerable amounts of endogenous N to the stomach contents. When a supplement of about 11 g of urea N was infused into the rumen little was retained in the digesta for the duodenal N flow increased by only 1 or 2 g, part of which was additional ammonia. As great an increase was brought about by feeding the supplement of flaked maize, which contained only 1 g of N. Various additions to the hay plus urea régime were tested. The infusion of 175 m-moles of a mixture of acetate, propionate and butyrate (65: 20: 15) had little effect on the duodenal N flow but infusion of the same amount of isobutyrate, 2-methylbutyrate and isovalerate (50: 25: 25) or feeding the flaked maize supplement increased the N flow by a further 1 or 2 g.

The increased flow of N brought about by these last treatments conforms with the experiments of Hemsley & Moir (1963) which showed that similar dietary supplements increase the amount of hay eaten by sheep and its digestibility. Nevertheless our supplemented urea treatments did not raise the duodenal N flow to nearly as high a value as was found when a similar N intake was provided by a diet of hay, flaked maize and soya protein (Kay & Phillipson, 1962).

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Effect of surplus leucine intake on serum cholesterol in man. By A. S. Truswell, M.R.C. Atheroma Research Unit, Western Infirmary, Glasgow, W 1

This investigation was prompted by two considerations. First, it has been reported that adults showed a fall of serum cholesterol when their dietary protein was reduced

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isocalorically to 25 g/day or less (Olson & Vester, 1960), and serum cholesterol is low in untreated kwashiorkor (e.g. Schendel & Hansen, 1958). Secondly, leucine is unique among the amino acids in that it is katabolized, via isovaleryl-coenzyme A, to β-hydroxy-β-methylglutaryl-CoA, which is a direct precursor of cholesterol. Furthermore, Zabin & Bloch (1950) found in rats that five times as much isotope appeared in cholesterol after giving carbon-labelled isovalerate as was formed from labelled acetate. It therefore seemed possible that leucine might be one factor determining the effect of dietary protein on serum cholesterol.

During experiments on the effects of excess leucine on human N balance (Truswell, 1962) and nicotinamide metabolism (Truswell, 1963; Truswell, Godsmith & Pearson, 1963) serum cholesterol levels were measured. Apparently normal adults received constant diets of whole maize meal (420-550 g/day), vitamins and minerals. Sucrose was added to maintain body-weight. A leucine supplement was given for 9 days; isonitrogenous amounts of glycine were given in control periods. Serum cholesterol was measured every 3rd day. The table shows means of three determinations before and three after leucine compared with means of the last two cholesterol values during leucine periods.

		Ser	um cholesterol (mg	100 ml)			
Subject	Control	Leucine	Control	Subject	Control	Leucine	Control
•	L-leucir	ne supplement	6 g/day	_	L-leucir	ne supplement	2 g/day
P.N.	123	127	113	P.N.	104	100	100
P.N.	113	124	115	J.M.	166	171	172
J.M.*	188	165	178	J.M.	182	179	165
M.P.*	179	169	183	M.P.	176	172	170
M.P.*	191	178	197	S.Z.	179	184	172
O.B.*†	216	185	189	S.Z.	172	157	169
Mean	168	158	162	J.P.	195	189	186
		_		Mean	168	165	162
		*Low ni	cotinamide intake.	†N	Mixed diet.		

Serum cholesterol was not elevated by adding leucine to diets already containing a high proportion of leucine.

Most of the subjects were studied in the Department of Medicine, University of Cape Town (supported by C.S.I.R. & U.S.P.H.S. A3995). I am grateful to Dr B. Bronte-Stewart for cholesterol determinations.

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