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ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Thirty-sixth Scientific Meeting of The Nutrition Society was held in the Atkins Building, Queen Elizabeth College, Campden Hill, London W8 7AH, on Friday, 24 September 1971, at 14.30 hours, when the following papers were read:

Relative constancy of urinary creatinine and urochrome. By K. S. ISMAIL, M. A. KHAN and A. E. BENDER, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Because of the difficulty of obtaining with certainty a 24 h sample of urine, the estimation of urinary constituents is often related to the creatinine output. This is based on the finding that creatinine excretion is related to muscle mass and independent of diet (Folin, 1905). However, recent work shows that the daily output of creatinine can vary considerably, even in the same individual (Paterson, 1967).

Since the output of urochrome, the principal pigment of normal urine, is related to general metabolism and is said to be constant for an individual (Drabkin, 1930), the urinary excretions of creatinine and urochrome were compared.

Twenty-four hour samples of urine were collected daily for 6 successive days from twenty-five subjects (nineteen females and six males). Creatinine was estimated by Jaffe's picrate method and an index of the urochrome was obtained on an arbitrary scale by comparing the urine colour with standard solutions of potassium chromate.

The constancy of the daily output of each subject was estimated by averaging the standard deviations and coefficients of variation of the six successive daily values for each subject. The results were: urochrome, mean SD $\pm$54, mean CV 19·7; creatinine, mean SD $\pm$170, mean CV 16·6.

The over-all constancy of output was then estimated by calculating the SD and CV of the mean of the mean daily output values of each subject. The results were: urochrome, mean 293 units, range 135–470 units, SD $\pm$58, CV 20; creatinine, mean 1145 mg, range 571–2119 mg, SD $\pm$335, CV 29.

The results indicate that for one individual daily creatinine output is less variable than urochrome and that, as a general measure for any individual, urochrome is more constant than creatinine. The procedure of basing urine analyses on creatinine or colour excretion on the assumption that the 24 h output of these index substances is constant leads to an error of the order indicated by the coefficients of variation above.

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The effect of deep-fat frying on the availability of fish lysine. By P. J. TOOLEY (introduced by K. J. CARPENTER), *St. Osyth's College, Clacton* and *Food Science Laboratories, University of Nottingham*

Little is known of the effect of thermally oxidized cooking oils on the protein quality of fried foods. Lea, Parr & Carpenter (1960) and Carpenter, Morgan, Lea & Parr (1962) suggested that the loss of 'available' lysine in stored herring meal was due to binding reactions between the ϵ-amino group of the lysine and carbonylic oxidation products present in the fish oil. The present investigation has been to study possible losses in available lysine during deep-fat frying of fish.

Filleted 10 g samples of fresh and frozen fish were fried in fresh samples of maize oil (A) and groundnut oil (B) for 4 min at 180°. The macerated fried fish was then extracted with light petroleum for 2 h. The samples were air-dried at room temperature overnight and their FDNB-available lysine content was determined (Carpenter, 1960). The experiments were then repeated with oils A and B after they had been used for continuous frying at 180° for 48 h. Lysine values were also determined for unfried control samples of fish and these agreed quite well with values abstracted from the literature (FAO, 1970). The results were:

	Fresh oil		Abused oil		
Characteristics of oils	A	B	A	B	
Iodine value (Wij)	125·0	99·0	94·0	75·0	
Peroxide value (mequiv. peroxide/kg oil)	2·0	2·5	4·5	4·0	
Linoleic acid (% methyl ester)	58·0	23·0	43·0	13·0	
FDNB-available lysine (g/16 g N) of fish fried in the oil:					Unfried control
Cod*	7·8	8·0	7·1	7·0	10·4
Haddock*	9·0	9·5	7·7	8·4	10·8
Coley*	9·7	10·4	8·5	8·6	10·8
Plaice*	8·5	9·3	7·4	8·0	10·1
Skate†	9·5	10·2	7·0	8·0	12·4
Rock salmon†	9·1	9·3	8·0	8·4	11·8
Mean for all species	8·9	9·4	7·6	8·1	11·0

Wij, Wijinski units.
*Mean of results using both frozen and fresh fillets.
†Mean of results using fresh fillets only.

The loss of approximately 17% available lysine during deep frying in fresh oil, compared with a loss of approximately 25% when using thermally abused oil, indicates lysine-binding reactions of the type already described. The losses are probably much higher than would be experienced in normal domestic and commercial practice because of the small sample size and the absence of crumb or batter coatings.

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The effect of diet on proteolytic activity in rat skeletal muscle. By D. J. MILLWARD,* (introduced by W. P. T. JAMES), *Tropical Metabolism Research Unit, University of the West Indies, Jamaica*

There are several reports describing the activities of lysosomal (Weinstock & Iodice, 1969) and alkaline (Pennington, 1970) proteolytic enzymes in dystrophic muscle. Few attempts, however, have been made to measure these enzymes in malnourished animals. This report describes the effect of a protein-free diet, starvation and refeeding on acid and alkaline proteolytic activity in rat skeletal muscle.

Rats were weaned directly on to a protein-free diet which was given for up to 24 d. In our experience, apart from the obvious weight loss, the rats were alert and active for the first 21 d of this regimen but deteriorated rapidly after this time. Refeeding after 24 d was achieved by the careful administration of milk and standard laboratory chow. Groups of animals were killed after 21 and 24 d on the protein-free regimen and after 4 and 7 d of refeeding. In addition, the effects of starvation were measured on 70–80 g male rats starved for up to 4 d. Groups of these rats were killed daily. In all experiments control rats were given the standard laboratory chow. Proteolytic activity was measured in a homogenate of gastrocnemius muscle at pH 3·8 using haemoglobin as a substrate. Autolysis was measured at pH 9. The results are shown in Table 1. There was no significant change in the proteolytic activities after 21 d on the protein-free diet. After 24 d, however, there was a small increase in the autolytic activity and a 100% increase in the activity at pH 3·8. On refeeding there was a significant reduction in the levels of activity of both enzymes. Starvation had no effect on the level of activity of either enzyme.

Table 1. *Effect of diet on acid and alkaline proteolytic activity in rat skeletal muscle*

		Proteolytic activity (% of control ± SD)	
Dietary regimen	Day	Alkaline (pH 9)	Acid (pH 3·8)
Protein-free	21	91·5±23·2	111 ±29·8
	24	129 ±15·4*	206 ±12·2**
Refeeding	4	44·5± 6·85**	60·5± 4·4**
	7	64·5±14·5**	62 ±10·5**
Starvation	1	100 ±12·9	115 ±10·9
	2	87·5± 4·97	104 ±17·4
	3	107 ±25·2	101 ±11·9
	4	86 ±10·3	88·5±15·25

Significance of difference (t): *$P<0.01$; **$P<0.001$.

These results suggest that there is no obvious connexion between muscle protein wasting and proteolytic activity, at least until the animal is in a terminal state. On refeeding, however, the rapid growth achieved by skeletal muscle may be related to a reduction in protein catabolism.

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The effects of sucrose-containing diets low in protein on ocular refraction in the rat. By M. BARDIGER and ANNE L. STOCK, *Department of Nutrition, Queen Elizabeth College, London W8 7AH.*

In our hands, simple deficiency of protein did not affect ocular refraction in the rat (Bardiger, Miller & Nicholson, 1968). In human situations such as urbanization in developing countries, diets frequently contain increasing amounts of sucrose as well as being low in protein. We have therefore repeated our experiments on growing rats with diets that have both of these characteristics.

In one experiment, control weanling female rats of the Sprague–Dawley strain were given stock diet (Amvilac) *ad lib.*, and three other groups diets containing 20% Amvilac with 80% starch, or sucrose, or glucose. Growth was retarded in the three experimental groups. After 202 d, a significant lowering of the normal hypermetropia (that is, a development of relative myopia) was seen in the rats receiving sucrose (Table 1).

Table 1. *Change in refraction (mean dioptres) in rats given various diets for 202 d from weaning*

Diet	Dioptres	SD	No. of rats
Stock	0·98	0·35	5
Starch	0·58	0·26	6
Glucose	0·40	0·34	5
Sucrose	0·125	0·31	5

In another experiment, three groups of weanling rats were fed as follows: group 1: diet A—Amvilac *ad lib.*; group 2: diet B—Amvilac with 80% sucrose; group 3: diet C—Amvilac restricted in amount so as to give a growth rate equal to that of group 2.

After 112 d the diets of groups 2 and 3 were reversed. Sucrose from weaning produced no change in refraction within the first 65 d but resulted in myopia within 105 d; this was not reversed when rats were transferred to the restricted but sucrose-free diet (Table 2). The restricted diet given from weaning produced no change in refraction by 105 d, but the change-over to the diet with sucrose resulted in the rapid production of myopia within the next 21 d.

Table 2. *Change in refraction (mean dioptres) in rats given various diets from weaning (six rats/group)*

Group..	1		2		3	
Diet..	Stock		Sucrose		Restricted stock	
Day of expt	Dioptres	SD	Dioptres	SD	Dioptres	SD
65	1·25	0·49	1·15	0·41	1·26	0·51
105	1·04	0·32	0·05	0·19	1·17	0·27
112 (diet: changed)	Stock		Restricted stock		Sucrose	
133	1·02	0·18	0·15	0·16	0·16	0·26
163	0·87	0·32	0·30	0·14	0·59	0·24
224	1·25	0·34	0·47	0·16	0·56	0·20
275	1·16	0·46	0·19	0·19	0·46	0·44

Thus, the eyes of both weanling and older rats are sensitive to the effects of sucrose in protein-deficient diets.

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Some effects of glucose syrup ingestion during vigorous exercises of differing intensities and duration. By L. F. GREEN, *Research and Development Department, Beecham Products, Great West Road, Brentford, Middlesex* and V. THOMAS, *Human Performance Laboratory, St Mary's College, Twickenham, Middlesex*

The effects of ingestion of an experimental glucose syrup drink (containing 46%, w/v, glucose syrup and mineral salts) were studied in physical education students. Blood glucose concentrations were measured by auto-analysis of capillary samples taken from a finger-prick (Morley, Dawson & Marks, 1968). Heart rate was recorded from cardiac telemetry and manual palpation (Thomas, 1970). Exercise, by ergometer cycling, treadmill running and circuit training (Morgan & Adamson, 1961), was taken (1) where subjects had fasted for 15 h, before continuous submaximal (aerobic) running or cycling for 60 min, or (2) for three periods of 4 min of maximal (anaerobic) gross muscular exercise, with 41 min rest between each work period.

Glucose syrup ingestion followed three routines: (1) one intake of 150 ml taken 15 or 45 min before exercise; (2) one intake of 150 ml taken 30 or 45 min before exercise followed by three intakes of 50 ml at 30 min intervals; (3) one intake of 150 ml taken 20 min before exercise followed by three intakes of 50 ml at intervals of 45 min.

All conditions were compared with similar conditions with a placebo and with no exercise. The order of subjects, conditions and trials was according to a Latin square. Some preliminary results have been reported elsewhere (Thomas, 1971).

The results are illustrated in Fig. 1. Glucose syrup ingestion caused a rise in exercise blood glucose levels ($P<0{\cdot}001$) with a time-lag and magnitude specific to

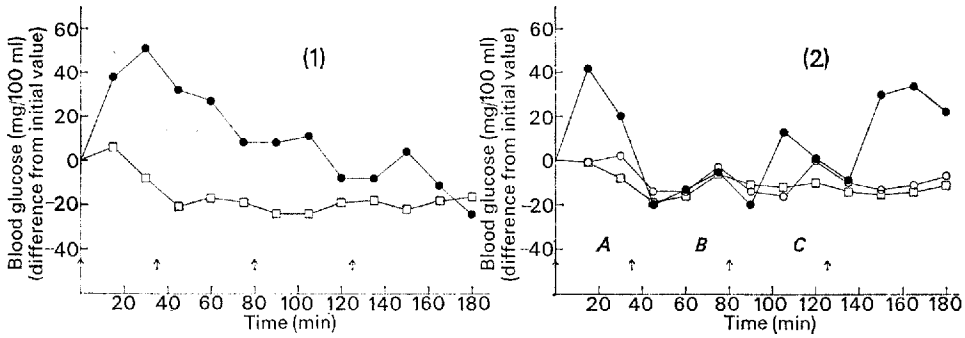

Fig. 1. Mean blood glucose concentration (difference from initial value) of (1) five subjects at rest and (2) five subjects at exercise. † denotes the taking of a drink; *A*, *B* and *C* are three periods of 4 min maximal gross muscular exercise; ●——●, drinking glucose syrup; □——□, taking a placebo; ○——○, taking no drink.

each individual subject. The elevation of blood glucose caused by glucose syrup ingestion was maintained during moderate and severe exercise of various types, and during the hour after exercise ($P<0.001$). Concurrently with the elevation of blood glucose, there was an improvement in the performance of both submaximal ($P<0.001$) and maximal ($P<0.05$) exercise, in terms of increased work done and decreased heart rate during the work.

Thanks are extended to the Research and Development Department of Beecham Products, who performed the chemical analyses and provided the experimental drink.

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Lactose intolerance in Kenya. By R. LUYKEN and F. W. M. LUYKEN-KONING, *Medical Research Centre, Nairobi, Kenya, Department of the Royal Tropical Institute, Amsterdam and Central Institute for Nutrition and Food Research TNO, Zeist*

Out of forty-five African children from a boarding-school near Nairobi, twenty-eight showed a biochemical lactose-intolerance, i.e. their blood glucose increased by less than 20 mg/100 ml after oral loading with 2 g lactose/kg body-weight. Symptoms after lactose loading were not reported. The average milk consumption was estimated at 250 ml/d.

With these children, the practical importance of lactose intolerance was investigated. For this purpose we traced in the first place how the proteins from the milk were absorbed by lactose-intolerant children ('non-absorbers'). The increase in serum α-amino acid nitrogen above the fasting level, after consumption of a standard quantity of milk, was taken as a criterion of absorption (Anfanger & Heavenrich, 1949; National Institute of Nutrition, Hyderabad, 1968). It appeared, that the increase of α-amino acid N in serum after loading the children with 340 ml milk at one time was the same in 'absorbers' and 'non-absorbers'.

We then investigated how well milk was tolerated by lactose-intolerant children, taking a lactic acid content of the faeces of more than 50 mg/100 g as criterion (Weijers & van de Kamer, 1965). Out of twenty-two children from 5 to 12 years old, who had received 250 ml milk, two had an increased lactic acid excretion. Six children from 5 to 15 years old were given 600 ml; only in two of them was the lactic acid excretion slightly increased. Also thirty-five children, aged 1–3 years, recovering from malnutrition, were examined. They consumed 250–500 ml milk. In 17% of them an increased lactic acid excretion was found. The majority of the children examined had no increased lactic acid excretion and thus tolerated the milk well.

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An explanation for the hypertriglyceridaemia of pregnancy. By D. P. RICHARDSON and D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Several animal species, including man and the rat, display a marked hypertriglyceridaemia during pregnancy. The rise in plasma triglycerides in the rat occurs during the last week of pregnancy (Hamosh, Clary, Chernick & Scow, 1970). Since the plasma triglycerides originate in the liver (Byers & Friedman, 1960), an increase in the plasma concentration could result either from a rise in the input from the liver, or from a fall in the rate of removal from circulation. The latter hypothesis was examined by Otway & Robinson (1968); a reduction in the activity of the plasma-clearing lipase was noted after the 19th day of pregnancy, but this occurred some 5–7 d after the triglyceride concentration began to rise. The work of Hamosh *et al.* (1970) confirmed this observation.

Naismith (1966) found an increase in the synthesis and deposition of neutral fat in the liver of the rat in late pregnancy. Furthermore, a positive correlation has been shown to exist between the degree of hepatic lipogenesis and the plasma triglyceride concentration in rats given high-fat or high-carbohydrate diets (Naismith, 1971). It seemed to us, therefore, that a rise in hepatic lipogenesis might explain the hypertriglyceridaemia of pregnancy.

The activities of three key enzymes involved in fatty acid synthesis were measured in the livers of eight pregnant rats, and of eight non-pregnant litter-mate controls, after 14 d of pregnancy, and in another eight pairs after 20 d. The changes found in enzyme activity (measured as i.u./liver) resulting from pregnancy were:

Enzyme	Percentage change in activity after 20 d
Pyruvate kinase	+56*
Glucose-6-phosphate dehydrogenase	+140**
Fatty acid synthetase	+67**

*$P<0.01$. **$P<0.001$.

On the 15th day of pregnancy, i.e. before the plasma triglyceride concentration begins to rise, no change in the activity of any of the enzymes was detected. On the 21st day, all three enzymes showed greatly increased activity.

The increased capacity of the liver to manufacture fat in late pregnancy has been attributed to a lack of insulin, combined with increased glucocorticosteroid secretion (Naismith, 1966).

This work was supported by a grant from the Gerber Group, which is gratefully acknowledged.

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Adaptations in the metabolism of protein during pregnancy in the rat.

By D. J. NAISMITH and R. B. FEARS, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

During pregnancy in the rat, the metabolism of protein proceeds in two phases. In the early anabolic phase, protein is stored in the maternal tissues when competition from the foetuses is minimal; in late pregnancy, when the foetuses are growing rapidly, catabolism of the protein reserve occurs, irrespective of the protein value of the diet (Naismith, 1969).

In the present study, nitrogen balance was measured for 14 d in pregnant rats and in non-pregnant litter-mate controls. The animals were pair-fed on a diet containing 25% casein. After the 1st week of pregnancy, the pregnant animals retained progressively more N than did their non-pregnant controls. This was achieved by a significant reduction in both faecal and urinary N excretion. The improvement in the efficiency of protein utilization permitted the retention of twice as much N as was required for the enlargement of the uterus and for growth of the products of conception.

One mechanism whereby a greater proportion of the ingested amino acids would be made available for anabolic purposes might be the suppression of the activity of enzymes in the liver that are responsible for amino acid catabolism. This hypothesis was tested in an experiment in which the activity of argininosuccinate synthetase, an enzyme which controls the rate of urea production, was measured in pregnant and control rats after 14 and 20 d of pregnancy. The blood of these animals was assayed for free amino acids and urea. The results are summarized in the table.

Table. *Percentage change in the activity (i.u./liver) of argininosuccinate synthetase and in the concentrations (μmol/ml) of amino acids and urea in the plasma of the rat during pregnancy*

Time	Argininosuccinate	Amino acids	Urea
After 14 d	(9)−48·5	(10)+34·9	(10)—21·9
After 20 d	(8)−35·0	(6)+42·0	(6)—13·2

Figures in parentheses are numbers of pairs used.

After 14 d of pregnancy, the capacity of the liver to form urea was almost halved. The plasma amino acid concentration was substantially raised and the urea concentration was reduced. This reduction in amino acid catabolism persisted to the end of pregnancy.

Progesterone is probably the agent responsible for these changes. Hervey & Hervey (1968) demonstrated that the administration of progesterone to female rats promotes an increase in carcass fat and protein, which they have attributed to increased food consumption. In our N balance experiment, in which the animals were pair-fed, progesterone metabolites were measured in the urine. The reduction in N excretion by the pregnant rat coincided with a rise in the excretion of pregnanolone and pregnanediol, thus implying a direct action of this hormone in the metabolism of protein.

This work was supported by a grant from the Gerber Group, which is gratefully acknowledged.

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The lipid content of the aortas of rats given sucrose. By K. R. BRUCKDORFER, I. H. KHAN and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

There is little published on the production of atherosclerosis in experimental animals by dietary sucrose. Chevillard and his colleagues, quoted by Trémolières & Lavau (1969), have reported that rats given sucrose develop atheroma, the degree depending on the amount of sucrose in the diet irrespective of the amount of fat. We have given to male Sprague–Dawley rats diets with 1·6% or 20% fat, and containing starch or sucrose, and we have assayed a number of lipid components in preparations of intima and media of aortas, that is aortas free from adventitia.

In the first experiment, two groups each of eight litter-mate weanling male rats were fed on diets with 1·6% maize oil and 68% starch or sucrose. In the second experiment, four groups each of eight litter-mate rats were fed on diets with either starch or sucrose (50%), and with either 20% arachis oil or with 5% arachis oil and 15% hydrogenated coconut oil. In both experiments, the rats were killed after 160 d, the aortas removed, cleaned and weighed, and lipid-extracted (Folch, Lees & Sloane Stanley, 1957). The extracted lipids were separated into phospholipid and neutral lipid by silicic acid chromatography, and the neutral lipids further fractionated by thin-layer chromatography (Skipski, Good, Barclay & Reggio, 1968).

In both experiments, sucrose was found to increase significantly ($P \leqslant 0{\cdot}05$) the concentration of total cholesterol, and of the free and esterified fractions (Table 1).

Table 1. *Lipid content of aortas (mg/g wet weight tissue) of rats fed on diets containing starch or sucrose*

Expt no.	Dietary component	Cholesterol			Triglyceride	Free fatty acid
		Free	Esterified	Total		
1	Starch	1·17	0·47	1·64	1·76	0·012
	Sucrose	1·72	0·61	2·33	2·11	0·013
2	Starch + arachis	1·8	0·46	2·30	1·34	0·015
	Sucrose + arachis	2·0	0·52	2·50	2·06	0·010
	Starch + HCO	1·9	0·46	2·39	1·46	0·010
	Sucrose + HCO	2·0	0·50	2·52	2·18	0·009

HCO, hydrogenated coconut oil.

The increase in triglyceride levels in the first experiment did not reach the 5% level of significance, but it did so in Expt 2. The nature of the fat in the second experiment made no difference to the level of cholesterol or of triglyceride, and neither the nature of the fat nor the nature of the carbohydrate altered the content

of free acid in the aorta. Although these different dietary treatments produced no difference in the total amount of phospholipid in the aorta, there appeared to be a change in the proportion of some of the fractions, notably in the lysolecithin: lecithin ratio. Further work is needed, however, before we can be certain whether these differences are real.

K.R.B. holds a Lord Rank Research Fellowship.

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Does dietary lactose produce hyperlipaemia in the rat? By K. R. BRUCKDORFER, S. S. KANG and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Fatty acid synthetase is increased in liver and decreased in adipose tissue, in rats when they are fed on diets containing a high proportion of sucrose or fructose rather than starch, glucose or maltose (Bruckdorfer, Khan & Yudkin, 1971). We have taken these results as implying an increase or a decrease in lipogenesis in these tissues (Chang, Seidman, Teebor & Lane, 1967; Saggerson & Greenbaum, 1970). We have now extended this to a study of dietary lactose. Because of the laxative effect of this sugar, we have used diets with no more than 30% lactose. At weaning, three groups each of eight Sprague–Dawley male rats were placed on diets with (1) 70% starch, (2) 40% starch and 30% lactose, (3) 40% starch and 30% sucrose. The rats in the lactose group had mild diarrhoea which disappeared after 2 weeks.

During the 8 weeks of the experiment, the group on the lactose diet, although consuming the same quantity of food, gained an average of about 230 g in weight, compared with about 275 g in the other two groups. Analysis of faeces showed that the digestibility of the lactose diet was 1–2% lower than that of the other diets, and analysis of urine showed a loss of some 3·5% of calories as galactose. These losses, together with that due to microbial decomposition in the enlarged caecum of the rats on the lactose diet, were no doubt responsible for the poorer growth.

Lactose did not affect the concentrations of plasma triglyceride or cholesterol compared with the concentrations in rats given the starch diet. The concentrations of synthetase in the liver in the groups receiving starch, lactose and sucrose were 0·64, 0·53 and 1·24 units/g tissue; in adipose tissue, the concentrations were 0·085, 0·061 and 0·086 units/g. Thus, lactose did not increase the activity of synthetase in hepatic tissue, nor greatly reduce the activity in adipose tissue; the small decrease in both liver and adipose tissue may have been due to the reduced rate and degree of absorption of this carbohydrate. As before, sucrose increased the activity of hepatic enzyme, though this time it did not decrease the activity of adipose tissue enzyme, perhaps because the diet contained less sucrose in this experiment. This

would also explain the failure of the sucrose diet this time to change the concentration of plasma triglyceride, although it did raise the concentration of plasma cholesterol.

K.R.B. holds a Lord Rank Research Fellowship.

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The hyperlipaemic effect of sucrose in male and female rats. By K. R. BRUCKDORFER, S. S. KANG and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Dietary sucrose produces a greater increase in plasma triglyceride in men than in premenopausal women (Macdonald, 1965) and in male than in female baboons (Coltart & Macdonald, 1971). We have carried out an experiment in rats to see whether they show the same sex difference. We took eight litters, each of two male and two female Sprague–Dawley weanling rats, and divided them into two groups of sixteen so that they contained equal numbers of litter-mate males and females. They were given a low-fat diet (1·6% maize oil) with 68% starch or sucrose. They were killed by bleeding after 16 weeks, and measurements were made of the level of cholesterol and triglyceride in the plasma, fatty acid synthetase activity in the liver, and lipoprotein lipase activity in the adipose tissue—epididymal fat pad in the males and perimetrial fat in the females.

The concentration of plasma triglyceride was higher in the male animals than in the female animals, but there was no sex difference in cholesterol concentration or in the activity of the enzymes (Table 1). In both male and female rats, sucrose produced

Table 1. *Plasma lipid and enzyme activity in male and female rats given diets containing starch or sucrose*

Dietary group	Plasma		Fatty acid synthetase	Lipoprotein lipase
	Triglyceride (mg/100 ml)	Cholesterol (mg/100 ml)	(units/g liver)	(units/mg N)
Male, starch	56	69	0·94	10·89
Male, sucrose	82·6	73	2·58	6·17
Female, starch	34	72	1·19	9·56
Female, sucrose	68·5	79	2·90	6·61

an increase in triglyceride concentration and in fatty acid synthetase activity, and a decrease in lipase activity. Thus, the lower concentrations of triglyceride in female animals cannot be explained by differences in the activity of these enzymes.

K.R.B. holds a Lord Rank Research Fellowship.

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The low-carbohydrate diet in the treatment of chronic dyspepsia. By JOHN YUDKIN and ELIZABETH EVANS, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*, and M. G. M. SMITH, *Liver Unit, Department of Medicine, King's College Hospital Medical School, London SE5*

Several investigators have shown that symptoms of chronic dyspepsia are not relieved by conventional dietary treatment with so-called 'gastric diets' (cf. Lawrence, 1952; Doll, Friedlander & Pygott, 1956). Consequently there has been a tendency to give only general dietary advice such as telling the patient to note and then to avoid foods known to cause indigestion and to eat small, frequent and regular meals.

One of us for many years observed that dyspepsia, which is commonly present in overweight patients, was considerably relieved during the course of treatment with the low-carbohydrate diet. We have therefore compared the efficacy of this diet with that of the simple dietary instructions now commonly used. Our subjects were men and women attending medical out-patient departments at King's College Hospital because of severe dyspepsia that had lasted for at least 6 months—in most instances for several years.

All patients were seen by one investigator for history and examination, and then passed to a second investigator for dietary advice. Alternate patients were placed on the 'orthodox regimen' and on the low-carbohydrate diet; after 3 months, their regimens were reversed. As far as possible, the investigation was conducted double-blind; except on rare and unavoidable occasions, the clinician was not aware of the treatment of any patient, nor the nutritionist aware of his progress.

The final assessment of the patient was made independently by two of us from the clinical notes; there was no disagreement as to the categorization of the patients into those in which the dyspepsia was better, not changed or worse with the low-carbohydrate diet compared with the orthodox regimen. At the time of writing, the numbers of patients in these categories were: improved 28 (19 male, 9 female); no change 11 (4 male, 7 female); worse 2 (1 male, 1 female).

We conclude that about 70% of dyspeptic patients are likely to benefit from a low-carbohydrate diet, compared with about 25% who would not be affected, and less than 10% who would feel worse.

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Experimental evaluation of anorexigenic agents in man: a pilot study. By A. D. MALCOLM, PAULINE M. MACE, K. P. OUTAR and G. L. S. PAWAN, *Metabolic Division, Department of Medicine, The Middlesex Hospital, London W1N 8AA*

This study was undertaken to compare the effects of the anorexigenic agents Fenfluramine, Phenformin, Diethylpropion and Phentermine on appetite, body-weight and plasma lipids in a virtually standard population.

Thirteen healthy male volunteers aged 19–28 years, all non-smokers, within 10% of their ideal body-weight and all without family history of obesity or diabetes mellitus, blindly received standard therapeutic doses of the four active preparations in random order, interspersed with four placebos indistinguishable from the drugs. Each subject ate *ad lib.*

During the first part of the trial the subjects received placebo for 1 week followed by 4 weeks on one of the active agents. After an interval of 5 weeks, subjects in the second part of the trial took each of the other three drugs for a fortnight, each period separated by 1 week on placebo. Fasting venous blood samples were taken on waking in the morning, twice a week, for measurement of plasma lipids; results are reported separately (Mace, Malcolm, Outar & Pawan, 1972). Appetite was scored according to a linear rating scale (Silverstone, Turner & Humpherson, 1968) and the subjects were weighed by a standard procedure on each day of blood sampling. Results are shown in Fig. 1. Similar results were obtained for the second

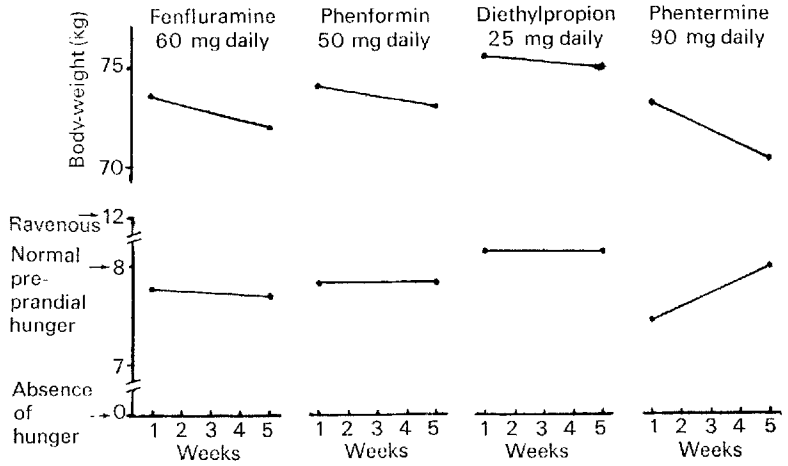

Fig. 1. Effects of anorexic 'antiobesity' agents on body-weight and 'linear appetite rating' in man (mean results).

part of the trial. There was no evidence of 'carry over' effects. Despite downward trends in body-weights clear suppression of 'appetite' by any of the agents was not seen, although there was a slight effect with Fenfluramine. There was no correlation between loss of weight and age, initial weight, height or percentage ideal weight. Our results suggest that appetite scoring should be cautiously interpreted, particularly as several subjects remarked that they had believed their appetites to be normal at the end of the first part of the trial but immediately upon stopping the drugs they noted exceptional hunger.

Side-effects were reported with both placebos and active agents. The mild somnolent effect of Fenfluramine and the stimulant effect of Phentermine featured prominently in the complaints and two subjects withdrew because of unacceptable stimulation with Phentermine.

We thank Dr R. G. Bird, The Medical Missionary Association and the subjects for their co-operation in this study.

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Comparative effects of four antiobesity agents on blood plasma lipids in man. By PAULINE M. MACE, A. D. MALCOLM, K. P. OUTAR and G. L. S. PAWAN, *Metabolic Division, Department of Medicine, The Middlesex Hospital, London W1N 8AA*

An earlier study had shown that the 'antiobesity' agent, Fenfluramine, produced changes in blood lipids in man (Pawan, 1969). We decided to investigate what effects other widely used 'antiobesity' drugs would have on blood lipids of a standard population. Because smoking, physical activity, sex, age, cold exposure, nutritional status, and other factors, may influence lipolysis (Pawan, 1971) and concentration of blood lipids, attempts were made to exclude or control these variables.

The experiment was conducted as described elsewhere (Malcolm, Mace, Outar & Pawan, 1972). Fasting venous blood samples were taken from the volunteers on waking in the morning, twice a week, and analysed by previously reported methods (Pawan, 1970). Results are summarized in Fig. 1. All four drugs appeared to affect

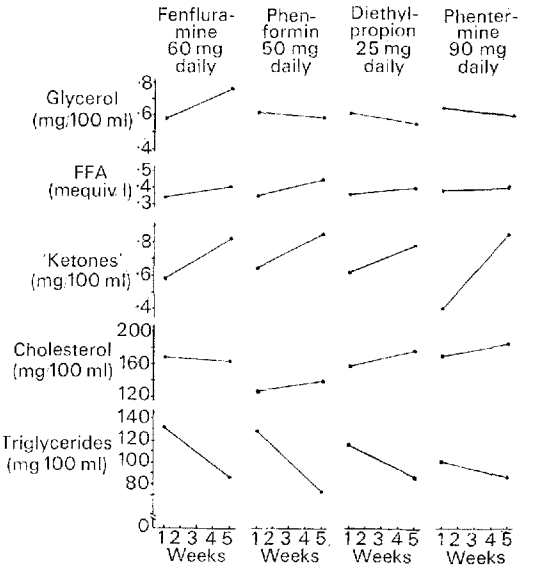

Fig. 1. Effects of 'antiobesity' agents on blood plasma lipids in man (mean values).

plasma lipids, decreasing triglycerides and increasing 'ketones' and free fatty acid concentrations. Fenfluramine produced an increase in free glycerol and a decrease

in cholesterol concentrations, whereas the other three drugs produced opposite effects on these measurements.

It is uncertain to what extent these results are primary effects of the drugs themselves, or secondary, due to other factors.

We thank Servier Laboratories Ltd, Merrell Division of Richardson-Merrell Ltd, Winthrop Laboratories, and Riker Laboratories, for supplies of 'antiobesity' agents.

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Food intake and weight maintenance. By M. J. STOCK, *Department of Physiology, Queen Elizabeth College, London W8 7AH*

Miller & Payne (1962) have demonstrated a large difference in the energy cost of maintenance between animals fed on high- and low-protein diets. McCracken (1968*a*) continued these observations when rats were fed *ad lib.*, but found that the differences disappeared when rats on both diets were 'meal-fed' by gastric intubation (McCracken, 1968*b*).

In the present work rats were fed on the same diets as used by Miller & Payne (1962) (designated HP and LP): (A) with no feeding pattern imposed; and (B) with one 2 h meal/d. The rats used for scheme B were pre-trained to the meal-eating regimen over a period of 5 d on stock diet before the experiment. This method of controlling meal patterns was considered less traumatic to the animals than feeding by tube. On each of the schemes A and B, twenty hooded rats aged 30 d were divided into four groups of equal weight, and two groups were placed on each diet. Rats on the LP diet were given excess food, whereas food to the HP groups was restricted in order to maintain the same weight as their respective LP groups. The results for energy intake and body-weight are summarized in Table 1.

Table 1. *Energy intakes and body-weights of rats on high-protein (HP) and low-protein (LP) diets*

	Scheme A		Scheme B	
	HP	LP	HP	LP
kcal/rat d	12·8	21·6	10·4	15·5
kcal/d $kg^{0.75}$	107	177	95	142
NDpCal/d $kg^{0.75}$	6·29*	7·11	4·25*	5·70
Mean body-weight during expt (g)	59·4	60·4	65·5	65·2
SD	1·8	2·1	1·9	1·6

*Values predicted from equation of Miller & Payne (1961).

The small standard deviations for mean body-weight indicate how well weight was maintained during the experiment. In each of the feeding situations, the LP groups

achieved this weight maintenance at a greater energy intake than the HP groups, and this was most pronounced in scheme A.

Carcass energy content was determined at the end of the experiment and it was possible to calculate differences in heat production between the LP groups and their respective HP groups. The LP rats on scheme A ate 8·8 kcal/rat d more than the HP rats, and of this 6·6 kcal (i.e. 75%) were lost as heat. On scheme B, the LP rats ate 5·1 kcal/rat d more than the HP rats and 3·8 kcal (i.e. 74%) were lost as heat.

Thus, the difference in the efficiency of energy utilization between the two diets was maintained in spite of the difference in meal pattern. Although the cost of weight maintenance was less with the meal-fed rats, it can be seen that it still depended considerably upon the composition of the diet.

I wish to acknowledge the advice of D. S. Miller on the conduct of this experiment and the assistance of R. Cox with the animals.

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Birth weight and growth rate during suckling and after weaning in strains of mice selected for mature body-weight. By MARGARET W. STANIER and L. E. MOUNT, *Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge*

Mice, like other species of animals, can be selected for mature body size, and this character is closely correlated with growth rate (Roberts, 1966). Studies were made on strains of mice selected over seventeen generations for high 6-week weight (L mice) and low 6-week weight (S mice), and on the randomly mated stock (C mice) from which the selection had been made.

The birth weight of the L mice was found to be greater than that of the S mice, but not significantly so, and was not due to a greater incidence of small size litters in this strain. When being suckled, growth rates differed markedly, mice in litters of natural size showing growth rates in the order L > C > S. In L and C mice, but not in S mice, growth rate during suckling could be increased by reducing the number of litter-mates to four.

To investigate maternal influences on growth rate during suckling, a group of four L mice were given on the day of birth to an S mother, and four of her young, born the same day, were reared by the L mother. The L young with an S foster-mother were still able to grow rapidly. Litters of S young reared by an L foster-mother showed diverse responses: some litters grew faster than they would have been expected to do with their own mother, others grew as slowly or more slowly (Brumby, 1960).

L and C litters, and most but not all S litters, showed a phase of rapid growth

after weaning, growth rates in this period also being in the order L>C>S. This phase was shown by L litters even if their growth rate during suckling had already been made unusually high by rearing in groups of four; this was not so with C litters.

It is concluded that the difference in growth rate between L and S strains during suckling is a property mainly of the young animals' appetite, and that the final adult body-weight is related to the extent of the rapid growth phase after weaning.

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A follow-up dietary survey of geriatric patients in hospital. By K. J. Acheson, Elizabeth Evans and Anne L. Stock, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

A previous survey (Evans & Stock, 1969) of geriatric patients in hospital revealed low dietary intakes, notably of energy, ascorbic acid and fat-soluble vitamins. A follow-up survey was requested by the hospital to assess the effectiveness of measures designed to improve intakes; these included better staffing, shorter times for vegetable preparation and daily provision of pure orange juice.

Intakes were assessed under the same conditions as before, for thirty-six women and eleven men (Table 1).

Table 1. *Daily nutrient intakes per head in three geriatric wards in the same hospital in 1969 and 1971*

	Rehabilitation ward		Medical ward		Long-stay ward	
	1969	1971	1969	1971	1969	1971
Energy (kcal)	1320	1425	1410	1145	1560	1100*
Protein (g)	41	51	49	42	61	42*
NDpCal%	9	9	9	9	10	11
Fat (g)	49	59	55	46	67	56
Iron (mg)	5·3	8·3*	8·1	7·6	8·5	7·1
Calcium (mg)	790	910	750	750	880	660*
Vitamin B_1 (mg/Mcal)	0·40	0·47	0·37	0·55*	0·45	0·57*
Vitamin B_2 (mg/Mcal)	0·82	0·82	0·71	0·93	0·78	0·96*
Nicotinic acid equivalent (mg)	13·1	15·5	11·7	14·1	17·2	15·3
Vitamin A (μg)	390	2230*	530	2110*	1050	1320
Vitamin D (μg)	0·65	1·44*	1·20	2·2*	2·80	2·26
Vitamin C† (mg)	7	29*	7	33*	9	38*
No. of patients	16	9	14	25	9	13
Age (years)	80	75	78	85	77	80

*Significant change ($P<0·05$).
†Determined by chemical analysis.

Energy intakes were not significantly changed in two wards but were lower in the long-stay ward, in which five patients were on reducing diets. The fall in energy

was accompanied by a fall in most other nutrients. The greatest increases were observed in ascorbic acid, vitamin A and vitamin D, and were due partly to increased consumption of green vegetables, fruit and eggs.

The orange juice supplied significant amounts of ascorbic acid (30%) despite the fact that it was sometimes not offered to, and occasionally refused by, the patients.

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Therapeutic hospital diets: as prescribed and as consumed. By G. JILL DAVIES, ELIZABETH EVANS, ANNE L. STOCK and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Individual weighed food intakes have been obtained for up to 7 d for thirty-two patients on special diets in two London teaching hospitals. Nutrient intakes have been computed from food composition tables (McCance & Widdowson, 1960). Intakes were compared with the diets as prescribed; errors inherent in the use of food tables have been assumed to apply both to the prescription and to the evaluation of the diets.

Table 1 gives the differences between the diets prescribed quantitatively and the diets as actually consumed, the latter including the food consumed by the patients from their own supplies. For patients on non-quantified reducing diets and low-fat diets the mean intakes are compared with those of age-matched controls from the same ward (Table 2).

Table 1. *Comparison of diets as prescribed and as consumed*

	No. of patients	Total days	Prescribed daily intake	Total intake	Intake from hospital supplies
Specified reducing diets:					
	2	14	300 kcal	230–1120 kcal	230–750 kcal
	5	31	1000 kcal	1010–1720 kcal	826–1350 kcal
	1	6	1800 kcal	2500 kcal	1910 kcal
Low-protein diets:					
	3	9	20 g protein	15–32 g	15–32 g
	2	9	40 g protein	61–88 g	61–81 g
Carbohydrate-controlled diets:					
	1	7	80 g carbohydrate	145 g	98 g
	4	28	120 g carbohydrate	134–219 g	83–146 g
	2	10	150 g carbohydrate	187–255 g	154–196 g
	1	7	200 g carbohydrate	226 g	224 g
Low-sodium diets:					
	5	12	480 mg sodium	629–6490 mg	380–2060 mg

Table 2. *Comparison of mean intakes of patients on special diets with those of controls*

No. of patients	Total days	Mean daily intake	Mean intake age control
Unspecified reducing diets:			
3 (60–69 years)	21	720–2110 kcal	1460 kcal
3 (80–95 years)	21	845–1430 kcal	1160 kcal
Low-fat diet:			
1	7	43 g	94 g

The intakes for most patients were quite different from recommendations. There were many examples of intakes of twice the prescribed amount and in one, a patient on a low-sodium diet, the intake was twelve times the prescribed amount. Some of the difference was due to the patients' own private supplies of food, over which there was no restriction: one patient on a low-salt diet received complete meals from his visitors. The ward staff also provided extra foods for the patients from the main ward supplies, in addition to the weighed diets supplied by the diet kitchens. For example, some patients on low-protein diets were provided with custard and ordinary bread, some on reducing diets were given thickened soups and bread, and some on carbohydrate-restricted diets were given porridge and bread.

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Fatty acid composition of triglycerides of lambs fed on barley-based diets.

By W. R. H. DUNCAN, E. R. ØRSKOV and G. A. GARTON, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Dressed carcasses from young lambs which had been fed for a few months on diets containing about 90% of rolled barley were found to have subcutaneous adipose tissue which was unusually soft. Samples, each about 10 g, of subcutaneous (rump) and perinephric adipose tissue were therefore taken from some of the carcasses for analysis of the fatty acids of their constituent triglycerides according to the procedure of Duncan & Garton (1967). The results of the analyses given in Table 1 relate to three lambs (A, B and C) which were given barley-based diets until they were slaughtered when 4 months old and, for comparison, to two year-old lambs (D and E) which had been fed on grass cubes.

Compared with lambs fed on grass cubes or sheep fed on conventional diets (Duncan & Garton, 1967), the subcutaneous triglycerides of lambs given barley-based diets contained considerably enhanced proportions of branched-chain acids and n-acids having an odd number of carbon atoms. The lower melting point of branched-chain acids relative to their straight-chain isomers (see Gunstone, 1967) probably accounts for the soft nature of these subcutaneous triglycerides, since their content of unsaturated fatty acids is not dissimilar to that present in the (firmer) subcutaneous triglycerides of grass-fed lambs. Although the perinephric triglycerides of the barley-fed lambs did not contain unusual proportions of branched-chain

Table 1. *Component fatty acids of triglycerides of perinephric and subcutaneous adipose tissue of lambs fed on barley-rich diets or on grass cubes*

(Values, to nearest whole number, as mol/100 mol total fatty acids)

	14:0	16:0	16:1	18:0	18:1	18:2	n-Acids with odd number of C atoms*	Branched-chain acids†
Barley-rich diets								
Lamb A								
Perinephric	4	27	2	19	35	5	4	2
Subcutaneous	2	23	1	10	42	5	6	9
Lamb B								
Perinephric	4	26	2	21	37	4	3	3
Subcutaneous	2	22	2	6	42	3	8	13
Lamb C								
Perinephric	4	26	2	21	38	4	2	1
Subcutaneous	4	23	2	6	39	5	8	13
Grass-cube diet								
Lamb D								
Perinephric	2	19	2	36	30	3	3	2
Subcutaneous	2	21	3	14	46	4	4	2
Lamb E								
Perinephric	2	20	1	30	32	5	3	2
Subcutaneous	3	22	4	9	47	5	3	1

*Mostly 15:0, 17:0 and 17:1. †Mostly monomethyl substituted 14:0, 15:0 and 16:0.

acids, they did contain somewhat more palmitic acid (16:0) and less stearic acid (18:0) than was present in the corresponding triglycerides of the older, grass-fed lambs and, in this respect, they resembled the perinephric triglycerides of young lambs reared on a lipid-free diet (Duncan, Garton & Matrone, 1971).

It was considered that the production of odd-numbered n-acids and branched-chain acids might have been promoted by the enhanced availability of propionate which results from ruminal fermentation of cereal-based diets (cf. Annison & Lewis, 1959; Ørskov, Fraser, Gill & Corse, 1971) and this supposition was subsequently confirmed (Garton, Hovell & Duncan, 1972).

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Effect of dietary propionate on the fatty acid composition of lamb triglycerides. By G. A. GARTON, F. D. DEB. HOVELL and W. R. H. DUNCAN, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

When lambs were given barley-rich diets, their triglycerides contained greater proportions of odd-numbered n-acids and of branched-chain fatty acids than are present in the triglycerides of grass-fed lambs (Duncan, Ørskov & Garton, 1972).

The possibility that enhanced availability of propionate is associated with this observation was investigated. The control diet contained rolled barley, 82%; soya-bean meal, 12%; white fish meal, 3% and barley straw, 3%; to this was added a mixture of vitamins and minerals. Acetate, propionate or butyrate (each as a mixture of anhydrous sodium and calcium salts in the proportions of 3 to 2 by weight respectively) was incorporated, at the expense of barley, as 21% of the metabolizable energy (ME) of the diet. Individually-penned male lambs were reared from about 20 to 50 kg over a 20-week period, during which their mean daily intake of ME from each diet was 2600 kcal.

Representative samples (each about 2 g) of the minced (dressed) carcasses of three control lambs and of three lambs given acetate, three given propionate and three given butyrate were taken for analysis of their triglycerides according to Duncan & Garton (1967).

Table 1. *Component fatty acids of carcass triglycerides of lambs fed on a diet containing 21% of the metabolizable energy as acetate, propionate or butyrate*

(Values, to the nearest whole number, as mol/100 mol total fatty acids)

Lamb no.		14:0	16:0	16:1	18:0	18:1	18:2	n-Acids with odd number of C atoms*	Branched-chain acids†
1	Acetate diet	2	24	3	19	41	5	3	2
2		3	27	2	19	43	4	2	1
3		3	24	3	19	41	5	2	1
4	Propionate diet	2	21	2	10	42	5	10	8
5		2	21	1	16	41	4	7	5
6		3	20	1	12	41	5	8	7
7	Butyrate diet	2	23	3	17	46	3	3	2
8		3	27	3	17	40	3	3	2
9		3	24	3	17	45	4	2	2
10	Control diet	3	24	3	13	45	6	3	3
11		4	24	3	12	41	6	5	4
12		4	26	4	10	41	5	6	3

*Mostly 15:0, 17:0 and 17:1. †Mostly monomethyl substituted 14:0, 15:0 and 16:0.

Table 1 shows that, whereas there was little difference between the groups in the proportions of 14:0, 16:0, 16:1, 18:1 and 18:2 in the triglycerides, the proportions of branched-chain acids and odd-numbered n-fatty acids in the triglycerides of the propionate-fed lambs were notably higher than those in the triglycerides of the lambs given acetate or butyrate; there was a corresponding reduction in the amount of stearic acid (18:0) present. A similar, though less marked, pattern was observed in the triglycerides of the control lambs.

While propionate is a known precursor of odd-numbered n-fatty acids (Horning, Martin, Karmen & Vagelos, 1961), its possible role in the biosynthesis of branched-chain acids in mammals is not so clearly established, though it could involve the

formation and utilization of methyl malonyl-CoA (cf. Cardinale, Carty & Abeles, 1970).

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Changes in the fatty acid composition of bovine adipose tissue associated with development. By G. A. EMBLETON and W. M. F. LEAT, *Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge*

As the rumen develops and the potential for rumen micro-organisms to hydrogenate dietary unsaturated fatty acids increases it might be expected that the stearic acid content of depot fat would increase to a plateau during the development of the ruminant. However, previous work (Leat & Embleton, 1970) suggested that in cattle this assumption was only partly correct and after 1 year of age depot fat became progressively more unsaturated. This period has been investigated in more detail by analysing samples of subcutaneous fat obtained by biopsy from Jersey cattle. Occasional samples of perinephric fat from slaughtered animals were also examined.

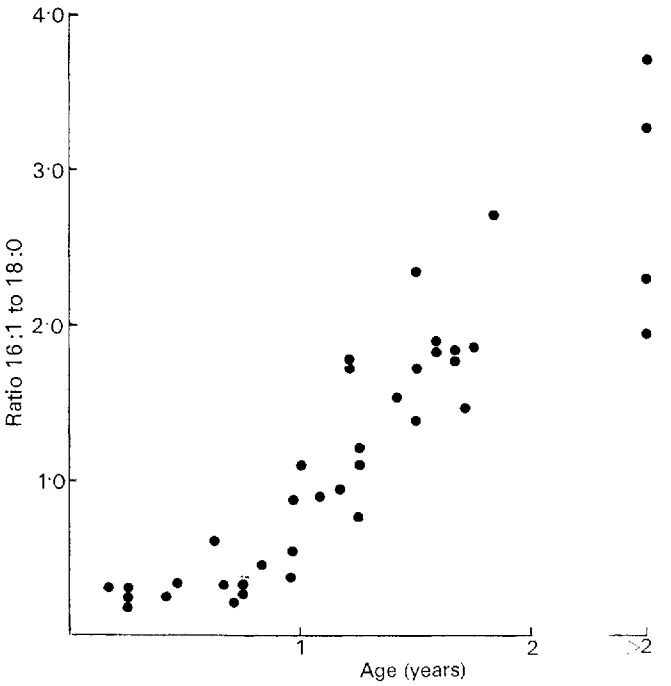

Fig. 1. Variation with age in the ratio of $C_{16:1}$ to $C_{18:0}$ in subcutaneous adipose tissue of Jersey cattle.

Changes in the percentage of $C_{18:0}$ acid were associated with inverse changes in $C_{18:1}$ acid in perinephric fat, and with $C_{16:1}$ acid in subcutaneous fat. The ratios

$C_{16:1}$: $C_{18:0}$ in subcutaneous fatty acids as a function of time are shown in Fig. 1. Values began to increase at 11 months of age and reached adult values at about 2 years of age. These changes appeared to be mainly independent of the sex of the animal. Evidence will be presented to suggest that the increasing unsaturation of depot fat may be associated with the fattening period of the animal, and may represent a desaturation of previously deposited fatty acids, or a progressive dilution of saturated fatty acid with newly formed acids of higher unsaturation.

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Retention of nitrogen by growing heifers given grass silage plus isocaloric supplements containing groundnut meal or urea. By T. W. GRIFFITHS, *The Agricultural Institute, Dunsinea, Castleknock, Co. Dublin* and I. H. BATH, *School of Veterinary Medicine, Trinity College, Dublin*

Balch (1967) has suggested that nitrogen retention in growing ruminants can be improved both by increasing the intake of energy, and of protein or non-protein N (NPN), when energy is not limiting. The experiment reported here investigated the response to additional energy and N using a basal diet of grass silage containing 54% of its N as NPN.

Eight Friesian heifers with an initial weight of 330 kg were used in a 4 × 4 Latin square experiment to assess the effects on N balance of supplementing a basal diet of grass silage with additional energy as barley and additional N as groundnut meal or urea. The basal silage, containing 0·32% N, was offered *ad lib.* either with or without 4 kg/d of a supplement based on barley with additional minerals and vitamins. The additional N was given either as 400 g extracted groundnut meal or as 60 g urea. Each experimental period consisted of a 20 d preliminary period followed by an 8 d period during which faeces and urine were collected and stored under acid. Metabolizable energy intakes were derived from urine and faecal energy losses and methane energy losses calculated using the equation of Blaxter & Clapperton (1965).

Metabolizable energy intakes were not significantly different on the supplemented treatments. N retention was significantly increased by supplementing the basal diet with barley and further significantly increased by the inclusion of both groundnut meal and urea.

Studies on rumen fermentation carried out at the same time showed that mean concentrations of ammonia N in rumen liquor were particularly low (about 5 mg/100 ml) on the barley and silage diet. Little NH_3 appeared to be produced by the rumen microflora from the protein of the basal silage, since most of the post-feeding increase was accounted for by the NH_3 present in the silage.

It is suggested that on the barley and silage diet microbial protein synthesis was limited by available ammonia N.

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Nucleic acids in ruminant digesta as indices of microbial nitrogen. By A. B. MCALLAN and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Our earlier observations (Smith & McAllan, 1970 and unpublished) indicated that dietary nucleic acids are rapidly degraded in the rumen and do not contribute appreciably to nucleic acid concentrations in the digesta. We have, therefore, used such concentrations, and mean values for microbial composition, to calculate the amounts of microbial nitrogen in calf digesta (Smith & McAllan, 1970, 1971). We have now studied microbial composition in greater detail.

In twenty-nine samples of mixed bacteria, separated by centrifuging rumen contents taken 4–6 h after six calves (14–42 weeks of age) had been given normal hay–cereal feeds, ratios (mean ± standard error) of RNA–N:total N and of DNA–N: total N were 0·114 ± 0·003 and 0·066 ± 0·004 respectively. Corresponding ratios were much lower in comparable groups of bacterial samples obtained from eight sheep (0·076 ± 0·003 and 0·051 ± 0·004) and nine cows (0·070 ± 0·004 and 0·051 ± 0·003). It is possible that the striking differences in properties of the bacterial populations between these groups were due, not to the different types of animals, but to the animals being reared in different environments. Corresponding mean ratios for thirteen different strains of rumen bacteria grown in pure culture were close to those shown by mixed calf rumen bacteria but with considerable individual variation (0·112 ± 0·007 and 0·074 ± 0·006).

Within each group (calves, sheep or cows) ratios similar to those shown above were obtained for animals at pasture and for those given all-roughage stall diets. Urea supplementation appeared to depress and a nearly N-free diet to elevate the ratios, but by only about 15–25%. Diurnal variations were apparent in all the stall-fed animals, and samples taken before a morning feed gave ratios about 20–35% lower than those shown above. Within each group, individual values for RNA–N: total bacterial N showed percentage standard deviations about one-half of those for the corresponding DNA ratios.

It is concluded that nucleic acid, and more particularly RNA, concentrations are suitable for estimating the contribution of total microbial N to rumen or duodenal digesta samples. A variable error is introduced by the presence of protozoa but this should normally be small (Smith & McAllan, 1970). It appears unnecessary to sample and analyse rumen bacteria in each experiment but calibration is necessary to establish bacterial composition for the particular animals and diet used in a particular environment.

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Effect of urea on digestion of fish meal in the gut of sheep. By E. R. ØRSKOV and C. FRASER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Previous work (Ørskov, Fraser & McDonald, 1971*a*,*b*) showed that with mature sheep increments of fish meal or soya-bean meal in barley diets caused increases

in the amount of protein passing the abomasum. However, with both protein sources large quantities were apparently degraded in the rumen. There is little potential for utilization of non-protein nitrogen (NPN), such as urea, when it is added to basal diets of barley containing 10–12% crude protein in the dry matter. With the young early-weaned lamb, responses in N retention have been noted to increments of urea up to a total of about 12% crude protein in the dry matter (Ørskov, Fraser & Corse, 1971). NPN is, however, often included as a part of protein supplements to ruminants and we have therefore examined whether urea, given together with a protein source, would have a sparing effect on the extent of rumen degradation.

Five sheep of approximately 40 kg live weight were used in a 5 × 5 Latin square design with five treatments consisting of a control diet and two fish-meal supplemented diets with or without urea. The results are given in Table 1.

Table 1. *Crude protein intake and disappearance in various segments of the digestive tract in sheep receiving rolled barley diets supplemented with either fish meal or combinations of fish meal and urea*

(Each value is the mean of five observations adjusted to equal dry-matter intake at 1025 g/d)

	Crude protein		Non-ammonia crude protein		Crude protein	
Treatment	Intake (g/d)	Disappearance in rumen (g/d)	Passing abomasum (g/d)	Disappearance in small intestine (g/d)	Disappearance in large intestine (g/d)	Excreted in faeces (g/d)
Unsupplemented	97	−33	131	80	10	44
Fish-meal level 1	124	−20	144	97	7	42
Fish-meal level 2	160	6	154	104	15	39
Fish-meal level 1 +urea level 1	163	21	142	96	12	37
Fish-meal level 2 +urea level 2	213	73	140	90	14	40
SE of treatment means	—	8·2	7·4	6·8	2·8	2·4

It can be seen that the only effect of urea supplementation was to increase the amount of crude protein removed from the rumen; this suggests that urea had no sparing effect on the partial degradation of fish meal which, calculated from linear regression, amounted to about 62%. The results suggest that inclusion of urea as part of a protein supplement in cereal-based diets is unlikely to be of benefit to the animal since the ammonia arising from the partial degradation of the protein supplement is supplying the NPN which may be required by the bacteria. If the protein supplement were protected from degradation, or given via the oesophageal groove, urea and protein supplements could then be used in combination, if the protein content of the basal feed were insufficient to meet the requirement of the rumen micro-organisms.

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The effect of urea supplementation on the nitrogen reaching the abomasum of lambs and on the extent of nitrogen recycling. By SARAH A. ALLEN and E. L. MILLER, *Department of Agricultural Science and Applied Biology, University of Cambridge*

There have been few quantitative studies of the conversion of urea into microbial protein in the rumen. Recently, Ørskov, Fraser & McDonald (1971) found that urea supplementation of a barley diet (9·5% crude protein) did not increase the amount of nitrogen absorbed from the intestine of adult ewes. We have investigated the effect of supplementing a basal diet containing 6·0% crude protein in the dry matter with 0·8, 1·6 and 2·4% urea on the N flow to the abomasum of ewe lambs.

Four lambs, initially weighing 15 kg and each fitted with a simple abomasal cannula, were given the four diets in turn at the rate of 26 g dry matter each hour. The basal diet was rolled barley 45·8, chopped straw 10, maize starch 30·9, molassine meal 10, vitamins and minerals 3, chromic oxide 0·3%. N flow to the abomasum was determined from the ratio of N to chromic oxide in dried digesta. Plasma urea entry rate was determined by isotope dilution (Cocimano & Leng, 1967). Urine was collected via an urethral catheter for the determination of urea excretion. The difference between urea entry into the plasma and excretion in the urine was assumed to be a measure of urea recycled to the rumen.

Table 1. *Effect of urea supplementation on urea metabolism and recycling and upon nitrogen passing through the abomasum (g N/24 h unless otherwise stated)*

Urea supplement (%)	Plasma urea (mg/100 ml)	Plasma urea entry	Urine urea excretion	Urea recycled	Dietary N ingested	Ingested + recycled N	Abomasal N flow
0	3·87	2·65	0·25	2·40	6·04	8·44	10·32
0·8	11·03	6·62	1·33	5·29	8·76	14·06	12·09
1·6	18·27	8·24	2·59	5·63	10·10	15·74	12·61
2·4	28·09	9·92	3·90	6·02	12·05	18·06	14·15
SE of mean	2·506	0·531	0·450	0·407	0·605	0·725	1·016

Results are given in Table 1. Increasing urea content of the diets resulted in a linear increase in flow of N to the abomasum ($P<0·05$). For all diets, abomasal N exceeded dietary N intake indicating the importance of endogenous N contributions. Plasma urea entry rate increased curvilinearly with urea concentration in the diet, whereas urea excretion increased linearly. Recycled N increased curvilinearly, with a large increase for the first addition of urea and little further increase with higher levels of supplementation. Linear regression analysis gave:

abomasal N flow = 0·48 (dietary N ingested) + 7·8,

abomasal N flow = 0·32 (N ingested + N recycled) + 7·7.

Comparison of these equations indicates the importance of recycling in increasing the effective conversion of urea N into microbial N entering the abomasum.

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The digestion of formaldehyde-treated groundnut meal before and after the abomasum of lambs. By E. L. MILLER, *Department of Agricultural Science and Applied Biology, University of Cambridge*

Formaldehyde treatment of groundnut meal has been shown to reduce ammonia production in vitro and to increase wool growth (Hughes & Williams, 1971). However, nitrogen retention was not affected whereas N digestibility was reduced. In the experiment now described the effect of a different formaldehyde treatment upon N flow to the abomasum and excretion in the faeces was investigated.

On the basis of in vitro results, 12 kg groundnut meal were mixed with 6 l of 2·4% (w/v) formaldehyde solution, spread on shallow trays and immediately dried at 60° overnight (15·5 h). Five lambs, fitted with abomasal cannulas, were given for 10 d a pelleted diet of ground straw 50, groundnut meal 25, rolled barley 19·7, trace minerals and vitamins 2, steamed bone-flour 2, urea 1, chromic oxide 0·3%. This was followed by three experimental periods, each of 4 d, during which the groundnut meal of the diet was in turn untreated, treated and untreated. Diets (1·5 kg/d) were given in two equal feeds each day. Flow of N and dry matter through the abomasum and excretion in the faeces were calculated by reference to chromic oxide concentrations.

Table 1. *Effect of formaldehyde treatment of groundnut meal on the extent and site of digestion of nitrogen and dry matter*

	Untreated	Formaldehyde-treated
N flow (% of N intake)		
Abomasum	71·7±2·01	99·1±2·01
Faeces	19·7±0·21	27·9±0·30
Absorbed post abomasum	52·0±1·92	71·1±2·71
Dry matter disappearance (% of intake):		
Prior to abomasum	29·4±1·30	21·2±1·30
Apparent digestibility	53·6±0·71	54·0±1·00
Post abomasum	24·3±0·97	32·9±1·36

The results are given in Table 1. Formaldehyde treatment increased the flow of N to the abomasum ($P<0·001$), increased the excretion of N in the faeces ($P<0·001$), and increased the N apparently absorbed post abomasum ($P<0·001$). Approximately 70% of the extra N reaching the abomasum was absorbed and 30% excreted in the faeces.

Formaldehyde treatment had no effect upon dry-matter digestibility but less was fermented in the rumen ($P<0·001$) and more disappeared between abomasum and faeces ($P<0·001$). The extra amount of dry matter disappearing in the intestines was twice as much as the extra weight of crude protein apparently absorbed. This would be consistent with formaldehyde treatment causing protection from rumen degradation of all components of groundnut meal and not of the protein fraction alone.

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Bacterial carbohydrates formed in the rumen and their contribution to digesta entering the duodenum. By R. H. SMITH and A. B. MCALLAN, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Three calves aged 14–42 weeks were given diets providing equal weights of flaked maize and hay. Six samples of mixed bacteria, separated by centrifuging rumen contents taken 4–6 h after feeding, were hydrolysed in 0·5M–H_2SO_4 for 4 h at 100°. Amounts of sugars liberated from the bacteria (g/100 g dry matter, mean values ± standard error), estimated by ion-exchange chromatography of their borate complexes (Smith & McAllan, 1971), were xylose (0·28 ± 0·05), arabinose (0·29 ± 0·10), rhamnose (1·00 ± 0·26), ribose (0·70 ± 0·09), mannose (0·42 ± 0·06), galactose (2·74 ± 0·86) and glucose (15·46 ± 2·75). Glucose liberated like this will be called 'starch'-glucose. 'Cellulose'-glucose (that liberated by a stringent two-stage acid hydrolysis) was present in only small amounts. Comparable samples (11) from sheep given diets fairly high in concentrate gave similar values except that 'starch'-glucose was lower (3·26 ± 0·62); this was still lower (1·56 ± 0·20) when concentrates were omitted from the diet. Replacing flaked maize by oats or barley in the calf diets did not affect bacterial carbohydrate composition but supplementation with urea depressed 'starch'-glucose by about 55%. 'Starch'-glucose values for samples taken from calves which had fasted for 16 h were about 30% of the post-feeding values.

Carbohydrate and nucleic acid analyses were carried out in nine samples of abomasal effluent taken 6 h after giving a calf diets containing equal weights of hay and cereal. Sugars present, largely in bound insoluble forms (g/100 g dry matter, mean values ± standard errors) were xylose (7·06 ± 0·94), arabinose (2·04 ± 0·25), rhamnose (0·46 ± 0·07), ribose (0·27 ± 0·04), mannose (0·17 ± 0·02), galactose (1·69 ± 0·21), 'starch'-glucose (9·42 ± 1·34) and 'cellulose'-glucose (10·78 ± 1·30). Related samples of rumen bacteria were similarly analysed and by assuming that these were representative of the rumen microbes and using nucleic acid as a microbial marker it was calculated that, in digesta entering the duodenum, nearly all the rhamnose, ribose and mannose and over half the 'starch'-glucose and galactose came from microbial synthesis whereas nearly all the xylose, arabinose and 'cellulose'-glucose came from the diet.

Thus it appears that rumen microbes may provide much of the 'starch'-glucose entering the duodenum particularly as glucose:nucleic acid ratios in protozoa are probably greater than those in bacteria. This agrees with the conclusions of Thompson & Hobson (1971) and suggests that variations in microbial carbohydrate composition, such as those shown above, could have a marked effect on an animal's nutrition.

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Carbohydrates entering the ruminant duodenum and their digestion.

By A. B. McAllan and R. H. Smith, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Sugars entering the ruminant duodenum are largely present in insoluble bound forms derived partly from the diet and partly from microbial synthesis. The amounts in samples of abomasal effluent taken 6 h after one calf was given a cereal–hay diet have been measured by Smith & McAllan (1972). A comparison of such samples from four calves showed little variation between animals in 'starch'-glucose values (mean value ± standard error was 7·68 ± 0·43 g/100 g dry matter) but bigger differences in corresponding values for xylose (7·28 ± 1·55), arabinose (2·68 ± 0·77), galactose (2·40 ± 0·77) and 'cellulose'-glucose (12·05 ± 3·33). These variations presumably represented mainly differences in the digestion of food constituents within the rumen and did not suggest marked differences in microbial carbohydrate composition. In other experiments, however, various factors led to differences in 'starch'-glucose in duodenal dry matter which corresponded to, and were most probably mainly governed by, variations in microbial composition (Smith & McAllan, 1972).

Thus 'starch'-glucose values for samples obtained after giving calves cereal–hay diets were higher than those for sheep given high-concentrate rations or for calves given a dietary supplement of urea. Samples from sheep given high-roughage diets showed even lower values and, in calves, a depression was found in samples taken after 16 h fasting. These different factors had variable effects on the other sugars present, ranging from increases when sheep were given high-roughage diets to decreases when calves were given a urea supplement.

In thirty-six experiments calves were given polyethylene glycol (PEG) as a non-absorbed marker with cereal–hay diets and the compositions of related duodenal and ileal samples compared. Calculated digestibilities of the carbohydrates present did not differ appreciably at different times after feeding (4 or 16 h). Mean (± standard error) 'cellulose'-glucose digestibility was 1 ± 4%. For the other sugars results were essentially the same whether PEG or 'cellulose'-glucose was used as the reference substance. However, the latter showed less variation and gave mean digestibilities of 5 ± 7, 80 ± 3, −1 ± 8, 10 ± 3, 5 ± 4, 12 ± 3 and 64 ± 3% for rhamnose, ribose, mannose, arabinose, galactose, xylose and 'starch'-glucose respectively. Apart from ribose, the digestibility of which approximated to that of RNA (Smith & McAllan, 1971), only 'starch'-glucose was removed to a great extent in the small intestine. Xylose and arabinose were apparently removed to a small extent but the mechanism is unknown.

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The Two Hundred and Thirty-ninth Scientific Meeting of the Nutrition Society was held in the Edward Lewis Lecture Theatre, The Middlesex Hospital Medical School, Cleveland Street, London W1P 7PN, on Thursday, 2 December 1971, at 11.00 hours, when the following papers were read:

The effect of litter size on subsequent energy utilization. By D. S. MILLER and SALLY R. PARSONAGE, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

The effect of litter size on growth in the rat was studied by Widdowson & McCance (1960). They showed that pups suckled in small litters were bigger at weaning than those in large litters. This difference was maintained throughout life, even though both groups were fed on a good stock diet *ad lib.* after weaning. The larger rats were also found to have a higher carcass fat-content. The reason for this difference in body-weight and composition could have been due to differences in food intake, or in efficiency of energy utilization, or both. The present work attempts to elucidate which of these factors was responsible.

By adjusting the number of pups per litter on the day of birth, several litters were produced varying in size from two to twelve pups. At weaning, some litters were killed for carcass analysis; the remainder were maintained on stock diet until 120 d old, when they were also killed for carcass analysis.

It was found that body-weight ($r = -0.8$) and carcass energy ($r = -0.9$) at weaning were inversely correlated with litter size. Similar correlations were found at 120 d old (see below). The total energy intake per rat from weaning (21 d old)

No. in litter	Body-weight (g) 120 d old		Body fat (%) 120 d old		Total energy intake (kcal)		Energy intake kcal/d $W^{0.75}$		Net efficiency (%)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
2	—	328	—	23·7	—	5030	—	199	—	47·6
4	475	257	22·0	22·6	6910	4820	238	205	49·4	39·3
4	447	316	16·1	20·6	6220	4850	209	203	50·1	43·8
8	428	259	17·7	18·2	6070	4450	208	208	47·6	38·8
10	373	228	13·7	17·3	6520	4320	197	183	41·0	39·3
12	328	214	11·6	13·3	5990	4140	243	225	22·6	23·1
r*	−0·96	−0·89	−0·81	−0·97	−0·57	−0·99	0·42	0·23	−0·86	−0·84
P†	0·01	0·02	0·1	<0·00001	>0·2	<0·00001	>0·2	>0·2	0·04	0·03

*Coefficient of correlation with litter size.
†Significance of correlation.

to 120 d old was inversely correlated with litter size, but when allowance was made for the differences in body-weight (kcal/d $W^{0.75}$) there was no correlation.

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Thus rats from smaller litters ate more, but this was due to their larger size, and the greater weight and carcass fat gain must have been due to an improved utilization of their energy intake. This is shown by the values of net efficiency (gain in carcass energy ÷ (energy intake − energy cost of maintenance)), which are inversely correlated with litter size.

A high plane of neonatal nutrition therefore appears to have metabolic effects apart from an increased appetite. This is consistent with the work of Knittle & Hirsch (1968) demonstrating marked hyperplasia of adipocytes after a high plane of neonatal nutrition.

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Effect of caloric density on energy utilization. By D. S. MILLER and SALLY R. PARSONAGE, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

The use of high-fat diets to make rats obese has been reported (Mickelsen, Takahashi & Craig, 1955) but there is little information as to whether these diets caused obesity by increasing energy intake, efficiency of energy utilization, or both. The effect of dietary fat and protein concentration on energy intake, energy utilization and the production of obesity is investigated in this work.

In the first experiment, weanling rats were fed *ad lib.* for 120 d on one of the three experimental diets, designated high-protein–high-fat, high-protein–low-fat and low-protein–high-fat. Food intake was measured and carcass energy gain was determined by the comparative carcass principle. In the second experiment, weanling rats were given isocaloric amounts of the three diets for 10 d and energy utilization was determined indirectly by the comparative carcass principle. The results for energy intake and utilization were:

	High-protein –high-fat diet	High-protein –low-fat diet	Low-protein –high-fat diet
Caloric density (kcal/g)	7·8	4·8	7·8
Protein kcal (% total kcal)	12	12	6
Expt 1 (120 d):			
Energy intake (kcal)	8100±283	7580±174	5190±271
Carcass energy gain (kcal)	1940±147	1700±110	750±128
Net efficiency of energy utilization (%)	74±2·8	85±4·7	36±8·6
Expt 2 (10 d):			
Energy intake (kcal)	970±32	970±35	970±31
Carcass energy gain (kcal)	200±13	160±8	90±11
Net efficiency of energy utilization (%)	44±4·7	45±3·0	24±5·2

The limits given are the standard errors of the mean.

Rats preferred the high-protein diets and, with *ad lib.* feeding, the gains in carcass energy were positively correlated with energy intake. However, the rats consuming the low-protein–high-fat diet had a significantly lower net efficiency of energy utilization than the other groups. When these diets were offered iso-

calorically, rats on the high-protein–high-fat diet still showed the greatest carcass energy gain and those on the low-protein–high-fat diet the least, and the net efficiency of energy utilization of the latter was still significantly lower.

In summary, rats fed on the high-protein–high-fat diet have shown maximum food intake concomitant with a high efficiency of energy utilization, thus suggesting that dietary protein concentration is as important as dietary fat in producing obese rats. This finding is supported by using the high-protein–high-fat diet to produce rats of 700 g body-weight and 40% body fat in 6 months.

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Seasonal variations in food intake in two Ethiopian villages. By D. S. MILLER and J. RIVERS, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Most discussions of the nutritional problems of the underdeveloped nations make two dubious assumptions: that the nutrient intakes of individuals can be meaningfully assessed by studying the food consumption of groups and that a nutrient intake below a stated minimum requirement is necessarily pathological.

Individual food intakes were weighed six times a year in each of two villages in Ethiopia. All food eaten by forty individuals over a 3 d period was measured on each occasion. The year was divided into two 'seasons'; one associated with harvesting, the other with the rains. The survey results obtained within each season were not statistically different and were therefore combined. Energy intakes between the two seasons were very different:

Average energy intakes (kcal) at two different seasons

	Adults		10–14 years		
Village	Males	Females	Males	Females	5–9 years, both sexes
		Harvest (October–February)			
Debark	1600	1200	1500	1100	800
Adi Arkai	1800	1000	—	1200	1000
		Rains (March–September)			
Debark	1200	800	1200	800	600
Adi Arkai	1300	800	—	700	600

Changes in food intake were not associated with changes in market price or food availability but were consistently associated with a change in dietary pattern. It seems likely therefore that the palatability of the diet was an important factor limiting food intake.

The energy intakes were extremely low, not more than that required theoretically for the basal metabolic rate. However, they were not associated with any catastrophic effects such as a decline in body-weight, reduced ability to engage in physical work, clinical signs of starvation, or a high death rate except with the under fives.

There may have been a reduction in the yield of breast-milk. It seems probable that energy expenditure was reduced both by a lowered work output and by a metabolic adaptation (Miller & Stock, 1969; P. Mumford, personal communication). The fitness of the adult population and the absence of clinical signs of protein deficiency indicate that impairment of nitrogen balance by low-energy intakes predicted by Miller & Payne (1961) does not occur in chronically undernourished subjects.

It seems that chronic undernutrition is not as pathological as many authorities suggest. One simple way of solving the world food problem might be to reduce the accepted norms for calorie requirements.

We are grateful to our colleagues on the International Biological Programme Ethiopian expedition for their assistance.

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The effect of diet and infection on creatine turnover in the rat. By J. C. WATERLOW and LUCY ROWE, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine* and R. E. NEALE and I. PALLIN, *Tropical Metabolism Research Unit, University of the West Indies, Jamaica*

In children with protein–calorie malnutrition, loss of muscle mass is probably the best index of the extent of protein depletion. The 24 h creatinine excretion is widely used as a measure of muscle mass (cf. Viteri & Alvarado, 1970). It is generally assumed that urinary creatinine is derived exclusively from muscle creatine and that about 50 mg creatinine corresponds to 1 kg muscle (Cheek, Brasel, Elliott & Scott, 1966).

The amount of creatinine excreted daily per g muscle depends upon the creatine content of muscle and the fraction of that creatine which is converted daily into creatinine and excreted. This fractional rate will be referred to as the 'creatine turnover rate'. The use of the same factor (50 mg creatinine/kg muscle) for calculating muscle mass from creatinine output under different nutritional conditions implies that creatine turnover rate remains unchanged. On general grounds this seems unlikely but, as there is no evidence on the point, experiments were done to test it.

Rats were injected with [1–^{14}C]creatine and, after 3–4 d for equilibration, urine was collected for 3 d and the radioactivity measured. The rats were killed; carcass, skin, viscera and a sample of muscle were extracted with 5% (w/v) trichloroacetic acid and the radioactivity was measured in the extracts.

Ninety per cent of the counts were found in carcass+muscle. It is supposed that the 10% of counts in viscera and skin are from muscle included in those tissue fractions. The creatine turnover rate averaged 2% per d in young growing rats on a normal diet. It was not significantly different in rats which were losing weight on a low-protein diet, or in older rats which were not growing. This rate is in close

agreement with that found by Bloch, Schoenheimer & Rittenberg (1941) with ^{15}N-labelled creatine. The turnover rate was increased in rats which were starved for 5 d and in rats which had a severe lung infection.

Measurements of non-isotopic urinary creatinine excretion are not reported here because the colour reaction is non-specific and the values are therefore less reliable than those derived from radioactivity measurements. No evidence was found of significant changes in muscle creatine content.

It is concluded from these preliminary experiments that the creatine turnover rate is rather constant, regardless of age, sex or dietary protein intake, provided that there is not severe or rapid loss of weight and muscle mass. Except in such conditions, creatinine output should provide a valid measure of muscle mass.

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Pantothenic acid deficiency in chicks fed on copper-supplemented diets.

By D. R. LLOYD and N. K. JENKINS, *Department of Physiology and Biochemistry, University of Reading*, and MARIE E. COATES and G. F. HARRISON, *National Institute for Research in Dairying, Shinfield, Reading*, and T. R. MORRIS, *Department of Agriculture, University of Reading*

Duplicate groups of ten chicks were fed from 1-d-old on diets based on wheat and fish meal, with or without 3·9% maize oil. The composition of the basal diet was similar to that described by Jenkins, Morris & Valamotis (1970) except that the maize oil was used in place of tallow. Comparable groups received the diets supplemented with 250 parts/10^6 copper as $CuSO_4 \cdot 5H_2O$.

Compared with their corresponding controls, at 4 weeks of age the chicks given Cu without maize oil were 7·6% lighter in body-weight, whereas those given Cu and

Table. *Expt 2: Effect of copper and maize oil supplementation of a wheat-based chicken diet on the growth of the chicks and on the copper and pantothenic acid contents of the diet and the chicks' livers*

Supplements to diet	Mean live wt at 4 weeks (g) (2 × 10)	Food consumption 0–4 weeks (g) (2 × 10)	Food efficiency*	Cu content (μg/g) Food (1)	Cu content (μg/g) Liver (5)†	Pantothenic acid content (μg/g) Food (1)	Pantothenic acid content (μg/g) Liver (5)†
None	315	570	0·553	6·4	4·10±0·50	10·8	69±2·3
Maize oil	336	583	0·577	5·6	3·95±0·46	9·7	71±7·3
Cu	308	550	0·560	261·6	5·06±0·90	10·9	67±8·3
Maize oil +Cu	184	386	0·477	250·5	11·40±4·48	7·1	53±9·3
SEM	8·2	14·7	0·013		1·03		4·32

Figures in parentheses are the numbers of observations.
*Weight at 4 weeks (g)/food consumption (g).
†Mean values and standard deviations for fresh liver.

maize oil were 55% lighter and showed signs characteristic of severe pantothenic acid deficiency. These signs were alleviated by two intramuscular injections of 1·5 mg calcium pantothenate given on successive days.

The results were repeated in a second similar experiment in which Cu and pantothenic acid analyses were made on the diets and on the chicks' livers. The results are given in the table.

The pantothenic acid in the diet appears to have been partly destroyed as a result of interaction between the maize oil and Cu supplements.

There was also evidence from further experiments that the effects of the addition of the copper sulphate supplement as crystals were more severe than those observed when it was added as a solution.

We are grateful to Mr K. J. Scott, National Institute for Research in Dairying, Shinfield, Reading, for the assays of pantothenic acid.

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Estimation of the fat-free mass of twenty subjects from measurements of total body potassium, body density, skinfold thickness, and height and weight. By J. WOMERSLEY, K. BODDY, PRISCILLA C. KING and J. V. G. A. DURNIN, *Institute of Physiology, University of Glasgow, and Scottish Research Reactor Centre, East Kilbride, Glasgow*

The fat-free mass (FFM) of ten male and ten female subjects was calculated from measurement of (1) total body potassium, using the factor 68·1 mequiv. K/kg FFM (Forbes, Gallup & Hursch, 1961) and, again, using the regression equations of Boddy, King, Hume & Weyers (1972); (2) body density; (3) skinfold thickness, and (4) height and weight, using the regression equations of Hume (1966) and Hume & Weyers (1971). All the subjects were apparently healthy and in the medium range of body build; all but two were aged between 17 and 32 years.

There was good agreement in the male subjects between the values for FFM from densitometry, ^{40}K by the formula of Boddy *et al.* (1971), and from the height and weight formula of Hume & Weyers (1971); mean differences were less than 1 kg. Larger discrepancies occurred with the other comparisons, especially in the women.

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The effect of a moderate dose of alcohol on oxygen uptake in mice. By R. SHRIMPTON (introduced by G. L. S. PAWAN), *Metabolic Division, Department of Medicine, The Middlesex Hospital, London W1N 8AA*

The effect of ethanol on metabolic rate in man and animals has been the subject

of investigation for many years. The published results are contradictory (Perman, 1962; Kalant, Hawkins & Watkins, 1963; Barnes, Cooke, King & Passmore, 1965). An attempt was made to throw further light on the subject by using the metabolic chamber (Dowsett, Kekwick & Pawan, 1963). A group of ten male mice, each weighing approximately 38 g and given a standard laboratory diet (diet 41B), was studied daily in the metabolic chamber maintained at a constant temperature of 29°. On different days the animals were injected intraperitoneally with either ethanol 2 g/kg, in 1 ml aqueous solution, or with 1 ml physiological saline solution, and oxygen uptake and RQ measurements were made for the next 6 h. The experiments were conducted between 10.00 and 16.00 hours each day to avoid discrepancies due to diurnal variation and exercise (Kekwick & Pawan, 1963).

Table 1. *Effects of intraperitoneal injection of saline, ethanol, and tri-iodothyronine on oxygen uptake, carbon dioxide output, and* RQ *of mice (values are means* ±SD)

Injection	No. of expts	ml O_2/g h	ml CO_2/g h	RQ
Saline	9	1·69±0·06	1·49±0·06	0·87±0·04
Alcohol 2 g/kg	11	1·93±0·05	1·46±0·06	0·76±0·02
Tri-iodothyronine 150 μg/kg	1	2·44	2·16	0·89

The results are shown in Table 1. The effect of injecting the mice with tri-iodothyronine (150 μg/kg) on the final 2 d of the experiment is also shown. A slight but significant increase (+14%) in oxygen uptake and a fall in RQ were produced by the alcohol at the dose used.

I thank Dr G. L. S. Pawan for facilities and encouragement.

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A semi-synthetic liquid diet as a way to administer ethanol to rats. By L. PILSTRÖM, E. FELLENIUS, I. BERGLUND and K-H. KIESSLING (introduced by G. L. S. PAWAN), *Institute of Zoophysiology, University of Uppsala, Uppsala, Sweden*

For ethanol administration to rats, Lieber, Jones & DeCarli (1965) and DeCarli & Lieber (1967) have introduced the use of liquid diets as the only source of food and fluid. Originally, amino acids were used instead of proteins but later the amino acids were exchanged for casein with the addition of cystine and methionine. When trying this diet we always found a sedimentation of the casein obstructing the feeding-tubes. To avoid this, other different sources of protein were tried.

Particularly suitable for making a stable liquid diet was a protein-enriched dried-milk powder. This dried-milk powder (S. 67; purchased from Semper AB, Stock-

holm) contains 67% milk protein, 20% milk sugars, 1% fat, 8% mineral salts and 4% water. The vitamin content is extremely low and can be neglected. The milk powder is suspended in water together with carbohydrates, fats, salts and vitamins to yield the semi-synthetic liquid diet. It can be stored at 4° for 3 d without obvious bacterial or fungal growth, but in the animal rooms it must be renewed every day in clean feeding-tubes.

In an experiment on the long-term feeding of rats with ethanol we have used this diet. The caloric composition of diets for two groups of rats was equal except for carbohydrates, which contributed 45% for the control group and 7·5% for the ethanol-consuming group. The latter group received 37·5% of its calorie intake as ethanol. The rats were fed *ad lib.* on the two diets. In the control group the mean daily consumption per rat was 120 ml during the experiment which lasted for 80 d. In the ethanol-drinking group the corresponding value was 50 ml during the first days, but increased to 80 ml within 20 d and remained at that level throughout the experiment.

The growth of the rats in the control group was normal with a K value of −3·28 (cf. Zucker, Hall, Young & Zucker, 1941). In the ethanol-treated group there was an initial weight loss of 10%, but within 20 d this loss was abolished and a normal but slower growth was obtained (K = −2·84).

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Activities of liver enzymes involved in lipogenesis after short- and long-term feeding of rats with ethanol. By E. FELLENIUS, U. NISSBETH, K-H. KIESSLING and L. PILSTRÖM (introduced by G. L. S. PAWAN), *Institute of Zoophysiology, University of Uppsala, Uppsala, Sweden*

The effect of acute and chronic feeding (0–80 d) with a liquid, slightly methionine-deficient diet containing alcohol on activities of hepatic enzymes related to lipogenesis has been evaluated. Carbohydrates were isocalorically substituted for ethanol in the control animals. The following enzymes were studied: malate dehydrogenase ($NADP^+$), citrate lyase and acetyl-CoA synthetase. The content of triglyceride in the liver was also estimated.

Malate dehydrogenase ($NADP^+$) is mainly concerned with the supply of $NADP+H^+$ for lipid biosynthesis (Wise & Ball, 1964). The citrate cleavage reaction, via citrate lyase, provides acetyl-CoA for the synthesis of fatty acids (Kornacker & Lowenstein, 1965). The activation by acetyl-CoA synthetase of acetate derived from ethanol oxidation can be an additional mechanism for supplying acetyl-CoA for hepatic lipogenesis (Lieber & Schmid, 1961).

The maximal concentration of triglycerides in the livers of animals kept on a liquid diet containing ethanol was reached after about 30 d and was at that time

almost three times as high as in control animals. The activity of malate dehydrogenase ($NADP^+$) and citrate lyase decreased significantly in the alcohol group compared to control rats within 10 d and remained low during the rest of the experiment (80 d). After 20 d the acetyl-CoA synthetase activity increased significantly in the ethanol-fed rats but fell subsequently to values similar to those in the control livers.

In summary, despite a pronounced increase of the amount of triglyceride in the livers of rats given a liquid diet containing alcohol, there was a dramatic decrease in the activity of enzymes (malate dehydrogenase ($NADP^+$) and citrate lyase) involved in lipogenesis. Furthermore, the almost unchanged activity of acetyl-CoA synthetase shows that the utilization of acetate, produced when ethanol is oxidized, is not stimulated by chronic feeding with ethanol.

The activity of malate dehydrogenase ($NADP^+$) and citrate lyase in rat liver is known to be related to the lipogenic capacity of this tissue (Kornacker & Lowenstein, 1965; Tepperman & Tepperman, 1964). For example, it has been clearly shown that in the rat the depression of hepatic lipogenesis is accompanied by a reduction in the activity of both malate dehydrogenase ($NADP^+$) and citrate lyase (Kornacker & Lowenstein, 1965; Tepperman & Tepperman, 1964). Therefore, the decrease in the activities of malate dehydrogenase ($NADP^+$) and citrate lyase demonstrates that the accumulation of fat in the liver after ethanol intake is not a consequence of a stimulated fatty acid synthesis.

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Effects of ethanol in neonatal lambs. By D. B. LINDSAY, P. J. BARKER, N. FREINKEL and R. W. WHITE, *Department of Biochemistry, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge*

Ethanol intoxication has been reported in newly born ruminants (Cunningham & Brisson, 1955; Abe, Morrill, Bassette & Oehme, 1971). We tried to produce this condition in order to determine whether ethanol produces a fasting hypoglycaemia as occurs in man, the dog and the rat (cf. Bleicher, Freinkel, Byrne & Seifert, 1964). In initial experiments, twelve lambs were bottle-fed four times daily on an artificial milk containing 6% lactose, 2% cream and 6% (low-fat) milk powder, beginning 24 h after birth. After 5 d, six lambs were switched to a diet containing 8% glucose and 6% milk powder, the remaining six receiving an unchanged diet. Unlike Cunningham & Brisson (1955), we found only occasional transitory concentrations of blood ethanol (<20 mg/100 ml) in both control and experimental groups.

In subsequent studies, seven lambs were fed, from 24 h after birth, three times daily on a ration of 8% glucose plus 6% milk powder. In all lambs, ethanol was produced after 2–3 d, the concentration in the abomasum after 5–6 d rising in several to more than 500 mg/100 ml and in all to more than 300 mg/100 ml. Ethanol was

shown to be produced specifically from glucose from a yeast fermentation (*Torulopsis glabrata*), as detailed elsewhere (White & Lindsay, 1971). Ethanol concentrations in the stomach, peripheral venous blood and urine were very similar, suggesting that it was probably metabolized rather slowly. In confirmation of this, liver alcohol dehydrogenase levels averaged $1{\cdot}6 \pm 0{\cdot}5$ (4) units/g, compared with 80 units/g in two adult sheep. In three lambs killed at 24 h after birth, before ethanol induction, the level was even lower ($0{\cdot}16 \pm 0{\cdot}1$ units/g). Moreover, plasma acetate concentration, which in adult animals infused with ethanol rose from about 1 mmol/l to 2·4 mmol/l, in lambs rose from 0·2 mmol/l to only 0·3 mmol/l.

Plasma glucose concentrations (16 h post-absorptive) showed no systematic change with increase in ethanol concentration (in the absence of ethanol the mean was 49 mg/100 ml, and in the presence of ethanol 59 mg/100 ml). Plasma lactate concentration tended to fall slightly in presence of ethanol (24 mg/100 ml to 15 mg/100 ml), whereas in man it normally rises. There was no effect on liver glycogen concentration (about 1·5 g/100 g liver) or liver fatty acid concentration (3·6 g/100 g liver). Histological examination of liver showed no apparent differences in affected and control lambs.

Although other explanations are not excluded, it seems probable that the absence of a significant effect of ethanol on gluconeogenesis stems from the low level of alcohol dehydrogenase.

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The recovery of markers in passage through the duodenum of the sheep.

By N. W. Offer, R. F. E. Axford and R. A. Evans, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor*

One of the criteria for the use of markers in nutritional studies is that they should be totally recoverable. Most workers have achieved quantitative recoveries of chromic oxide and polyethylene glycol in faeces, and this has led to their widespread acceptance and use. We have confirmed these findings.

The extension of the use of these markers to food in passage through the gut has also been practised, but the recoveries of markers from the duodenal digesta that have been reported have been of the order of 85% (cf. Bruce, Goodall, Kay, Phillipson & Vowles, 1966; Topps, Kay, Goodall, Whitelaw & Reid, 1968; MacRae & Armstrong, 1969; Nicholson & Sutton, 1969).

It is our contention that this shortfall in marker recovery arises from the brevity of the collection periods which include a time of stress to the animal before it has become adapted to the interference.

Chromic oxide and polythylene glycol were administered by a variety of methods to sheep fitted with re-entrant cannulas and given a constant ration twice daily.

By means of an automatic collection device (Axford, Evans & Offer, 1971) and using extended periods of collection, we have obtained quantitative recoveries of markers from duodenal digesta (Table 1). The standard errors quoted include day-to-day animal variation, as well as analytical error—which is the minor component.

Table 1. *Recovery of markers from digesta*

Method of marker administration	Percentage recovery of marker (mean ± SE)
Cr_2O_3 in diet	93·7 ± 9·6
Cr_2O_3 by paper once daily	96·9 ± 7·3
Cr_2O_3 by paper twice daily	99·3 ± 10·2
Cr_2O_3 by capsule once daily	98·0 ± 2·1
Cr_2O_3 by continuous infusion	88·8 ± 9·6
PEG in diet	96·0 ± 11·0
PEG by pellet once daily	101·7 ± 4·9
PEG by pellet twice daily	98·3 ± 7·1
PEG by continuous infusion	88·2 ± 10·0

PEG, polyethylene glycol.

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The diurnal flow of markers administered by a variety of methods to sheep. By N. W. OFFER, R. A. EVANS and R. F. E. AXFORD, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor*

We have investigated the diurnal flow of chromic oxide and polyethylene glycol through the proximal duodenum of the sheep when these markers are presented by a variety of methods. The sheep were given a constant ration at 12 h intervals. Samples representative of the total duodenal flow were collected continuously and were divided by a time-controlled collector into 3 h fractions. The collection was continued for 5 d after a 3 d equilibration period. The ratio of marker to dry matter was determined.

Our results indicate that when either marker is incorporated into the diet, or when chromic oxide-impregnated paper is administered at meal-times, the diurnal variation in this ratio is minimal. The once-daily administration of polyethylene glycol as a highly soluble pellet, or chromic oxide in a gelatin capsule, results in a pronounced diurnal pattern. Extreme examples of our findings are illustrated in Fig. 1.

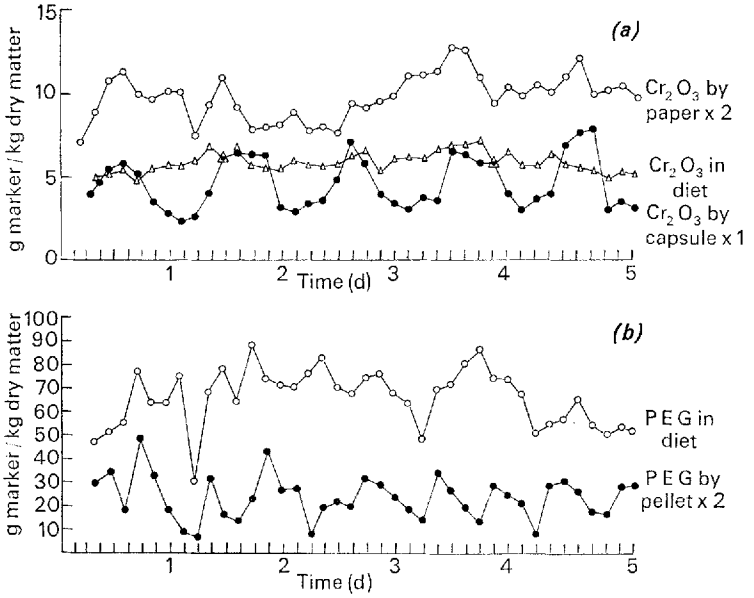

Fig. 1. Diurnal passage of markers. *a*, chromic oxide: ○—○, by paper; △—△, in diet; ●—●, by capsule. *b*, polyethylene glycol (PEG): ○—○, in diet; ●—●, by pellet.

No consistent diurnal pattern of dry-matter flow was observed; this was in accordance with the findings of Nicholson & Sutton (1969).

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The validity of marker practice for measuring the flow of digesta in sheep. By N. W. Offer, R. A. Evans and R. F. E. Axford, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor*

The commonest application of markers in the study of the flow of materials through the intestine is to correct for the depressed flow-rate induced by interference with the animal. The validity of this correction depends on two assumptions.

(1) The marker must be indigestible and, as a corollary, it must be quantitatively recoverable over a period of time at any transect of the gut.

(2) The marker must be evenly distributed in the digesta. As pointed out explicitly by Bruce, Goodall, Kay, Phillipson & Vowles (1966) and by Topps, Kay, Goodall, Whitelaw & Reid (1968), the concentration of marker and of other constituents of the digesta must not vary with flow-rate.

We have studied the validity of these assumptions. The first has already been discussed (Offer, Axford & Evans, 1972). For the second, we have compared the flow of marker and dry matter through the intestines of sheep for 3, 6, 12 and 24 h collection periods over a total of 5 or 6 d subsequent to a period of equilibration.

Methods of marker administration which produce distinctive diurnal patterns in the ratio of marker to dry matter do not agree with the second postulate. Serious errors may be avoided, however, if such marker techniques are used when the collection period corresponds to one, or a constant integral number of diurnal cycles. Those methods of administering markers which do not show a distinctive diurnal pattern, and which satisfy assumption 2, may be used for shorter periods of collection.

Table 1. *Coefficients of variation* (CV) *of regression of markers on dry matter*

Method of marker administration	CV for collection periods of (h):			
	3	6	12	24
Cr_2O_3 in diet	11·4	10·3	10·1	6·1
Cr_2O_3 by paper once daily	22·3	19·8	13·3	8·7
Cr_2O_3 by paper twice daily	14·1	13·0	11·5	10·9
Cr_2O_3 by capsule once daily	32·8	29·4	10·6	4·0
Cr_2O_3 by continuous infusion	27·8	26·8	25·2	18·6
PEG in diet	21·1	18·9	16·8	12·6
PEG by pellet once daily	51·9	41·4	35·8	11·9
PEG by pellet twice daily	46·8	34·8	20·9	10·0
PEG by continuous infusion	39·6	38·7	33·4	25·8

PEG, polyethylene glycol.

Our results (Table 1) show the coefficients of variation associated with the various methods of administration of two markers, chromic oxide and polyethylene glycol, for different periods of sample collection.

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The measurement of calcium absorption in the sheep by an infusion technique. By G. MOSELEY and R. F. E. AXFORD, *Department of Biochemistry and Soil Sciences, University College of North Wales, Bangor*

The method of Cooper, Levitan, Fordtran & Ingelfinger (1966) for studying absorption in the human small intestine was modified and applied to the study of calcium absorption in Welsh mountain sheep.

A triple lumen catheter was prepared by welding together two Ryle's gastroduodenal tubes, sizes 12 and 14, and a fine tygon tube, so that the largest tube projected 50 cm beyond the smaller Ryle's tube, which overlapped the fine tygon tube by 10 cm. The catheter was lubricated and inserted through the distal end of a pair of re-entrant duodenal cannulas until the shortest tube opened 30 cm inside the duodenum. Test solutions containing Ca with polyethylene glycol as a marker,

introduced through the tygon tube, flowed along the duodenum past the open ends of the Ryle's tubes. The intraluminal contents were sampled continuously by siphonage from the two Ryle's tubes 10 and 60 cm beyond the point of infusion. After a period for equilibration, the samples from these sites were retained for Ca and polyethylene glycol determinations. From the composition of the two liquids and the rates of infusion and siphonage, values were calculated for the rates of absorption or secretion of Ca over the 50 cm segment of gut.

For two normal sheep maintained under constant dietary conditions the rate of Ca uptake over the 50 cm of the small intestine under test was $1{\cdot}266 \pm 0{\cdot}040$ and $1{\cdot}194 \pm 0{\cdot}030$ mmol Ca/min.

After an 18 h intravenous infusion of calcium borogluconate, calculated to provide 500 mg Ca, the rate of Ca uptake from the gut in two sheep was in one instance diminished to 18% of the control value and, in the other, became negative. Alternatively, an intravenous infusion of tetrasodium ethylenediaminetetraacetate calculated to chelate 500 mg Ca over 18 h caused an elevation of the rate of Ca absorption to 135 and 150% of the control values; vitamin D administration virtually doubled the control rates.

The technique caused little discomfort to the animals and the results obtained were consistent and reproducible, and variations in the rate of Ca absorption were observed under different conditions.

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The dietary availability for sheep of the phosphorus in a heat–treated mineral phosphate containing aluminium and iron. By R. G. HEMINGWAY and JEAN M. HEMINGWAY, *Department of Animal Husbandry, University of Glasgow*

A series of feeding trials has been conducted to assess the nutritional value for sheep of the phosphorus contained in a product (Polyfos) (POLY), described as a double phosphate of calcium and aluminium, and obtained by heat-treatment of a mineral phosphate ore. The product is of somewhat variable composition and the sample we used contained 15·8% P, 5·5% Ca, 10% Al, 0·75% fluorine and 6·75% iron (mostly as iron oxide). Comparison was made with dicalcium phosphate (DCP) used at rates to supply equivalent amounts of total P.

In the first trial, six adult wether sheep were used in pairs in a 3×3 Latin square design and fed on a basal diet of 800 g sugar-beet pulp alone (0·09% P) or supplemented with 1·74 g P/d as either POLY or DCP. Urine collections were made during the second 7 d of each of the three 14 d feeding periods. Supplementation with DCP had significant effects in increasing urine P, reducing urine Ca and increasing blood P. POLY was without effect (Table 1).

In the second trial, groups of 6-week-old lambs (10 kg) were fed on a basal low-P diet (Hemingway, 1963) containing 0·12% P, 0·60% Ca and 16·5% crude protein,

Table 1. *Mean daily urinary excretion (mg) of phosphorus and calcium and mean blood P concentration (mg/100 ml)*

	Low-P diet	Low-P diet +POLY	Low-P diet +DCP	Significant difference (P=0·05)
Urine P	32·2	43·0	693·2	280·1
Urine Ca	20·9	19·0	2·5	10·4
Blood P	5·4	5·2	7·6	1·8

supplemented with 0·22% P given as either POLY or DCP. After 6 weeks the mean initial blood P concentration (6·3 mg/100 ml) had fallen to 3·3 (low-P) and 2·3 (POLY) and was 6·6 mg/100 ml for the DCP-fed lambs. Reduced growth was observed and clinical rickets (detected visually in most, by X-ray examination in all) was diagnosed in the low-P- and POLY-fed lambs. All the lambs given DCP were free of rickets.

In a third trial, two lambs from each of the groups that were fed separately in the second trial continued to be fed on the same diets in metabolism cages. Those lambs given DCP had markedly greater positive P balances than those who were given POLY (Table 2).

Table 2. *Phosphorus balances (g/d) of growing lambs (means of two closely similar values)*

	Low-P diet	Low-P diet +POLY	Low-P diet +DCP
Intake	0·94	2·64	2·90
Faeces	0·92	2·40	1·50
Urine	0·01	0·01	0·08
Balance	0·01	0·23	1·32

It is concluded that the P contained in this product has little if any value for sheep.

REFERENCE

Hemingway, R. G. (1963). *Proc. Nutr. Soc.* **22**, xvi.

Cellulose digestibility in lambs fed on milk and dried grass. By E. A. DOMINGO, J. H. TOPPS and G. PRATT, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

Six lambs were artificially reared from 7 d of age on a milk substitute. The milk substitute was provided in amounts which followed the yield of a ewe with either twin or single lambs. Three animals were allocated to each of the two levels of milk provided. Dried grass (*Lolium perenne* L.) was offered *ad lib.* from the 5th week of age and intake was measured daily. The lambs were fitted with abomasal cannulas at 3 weeks of age. Cellulose digestibility was measured at weekly intervals using the lignin ratio technique by analysing both abomasal and faecal samples.

Dry-matter intake, expressed as g/kg $W^{0·73}$, was significantly higher ($P<0·05$)

in lambs on the lower level of milk intake. There were no significant differences in digestibility of cellulose in either the reticulo-rumen or the whole gut between lambs on the different milk intakes. Results are shown in Table 1.

Table 1. *Digestibility* (%) *of cellulose in the reticulo-rumen and in the whole digestive tract* (*means for six lambs*)

Week	7	9	12	14
Reticulo-rumen	51	63	70	80
Whole digestive tract	73	75	82	85

In both the reticulo-rumen and the whole gut, cellulose digestibility was highly correlated with age ($P<0{\cdot}001$). The corresponding regressions were:

in the reticulo-rumen $y=29{\cdot}78+3{\cdot}58\,x_1$, $r=0{\cdot}68$;
in the whole digestive tract $y=60{\cdot}63+1{\cdot}70\,x_2$, $r=0{\cdot}71$;

where y=cellulose digestibility (%); x_1=weeks 5 to 14; and x_2=weeks 7, 9, 12 and 14.

The difference between the slopes of the two regressions was significant ($P<0{\cdot}05$), and represented the digestibility of cellulose in the lower gut, being 21% at the 5th week and only 5% at the 14th week of age.

It is concluded that the digestion of cellulose in the reticulo-rumen at an early age is less complete than that in the older animal but the extent of digestion in the lower gut partly offsets this difference. Total digestibility of cellulose should not be taken as an indicator of rumen development in young lambs.

The effects of additional methionine on the quality of leaf protein concentrates differing in nutritive value. By T. E. TRIGG and J. H. TOPPS, *School of Agriculture, 581 King Street, Aberdeen AB9 1UD*

The quality of leaf protein concentrates (LPC) and the effects of adding methionine to the LPC were measured in groups of four male Sprague–Dawley rats in a series of experiments. Leaf proteins were offered as the sole source of protein at a level of approximately 10% protein in a basal diet, based on that of Miller & Bender (1955). Net protein utilization (NPU) was used as an index of protein quality.

Methionine was added at four levels to diets containing a high- and low-quality LPC, namely wheat and slow-processed red clover. Methionine was added at only one level to the other two LPC (Table 1).

The NPU of the unsupplemented LPC diets showed that the proteins varied considerably in quality. Further analysis indicated that the high- and low-quality diets differed significantly ($P<0{\cdot}01$) in apparent digestibility of organic matter and protein, and in true digestibility of protein.

A high correlation between the total content of available sulphur-containing amino acids and protein quality was found ($r=0{\cdot}927$, $P<0{\cdot}01$). Large responses

in NPU and food intake were obtained when small supplements of methionine were added to any of the LPC. The NPU of all LPC was increased to approximately that of casein. A maximum response to methionine was achieved with the two LPC subjected to more detailed evaluation. Rats given the wheat LPC and the two higher levels of methionine showed a reduced food intake and NPU; whereas rats fed on the slow processed red clover LPC and on the highest level of the amino acid had a reduced food intake only.

Table 1. *Net protein utilization of leaf protein concentrates, each supplemented with methionine at various levels, compared with that of unsupplemented casein*

	Level of DL-methionine supplement (g/16 g N)					
Diet	0·0	0·3	0·5	0·7	1·1	1·5
Wheat	52·5*	68·1*	77·0	76·1	67·7	—
Red clover (slow-processed)	24·0*	58·5*	—	65·6	69·7	69·7
Red clover (quick-processed)	18·7*	63·0*	—	—	—	—
Lucerne	40·9*	62·9	—	—	—	—
Casein	67·8*	—	—	—	—	—

*Average of replicated values.

The results indicate that methionine is a factor limiting protein quality of all the LPC tested, and that dietary amounts of this amino acid may play an important role in the regulation of food intake by rats.

REFERENCE

Miller, D. S. & Bender, A. E. (1955). *Br. J. Nutr.* **9**, 382.

The effect on intake of concentrates of exchanging rumen contents between fed and unfed cows. By J. A. BINES, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

When mixed diets of roughage and concentrates are given to cows there is a steady decline in the importance of rumen distension in controlling intake as the proportion of concentrate in the diet is increased (Bines & Davey, 1970). It was concluded that some product of digestion limited the intake of high-concentrate diets, and measurements of acetate levels in blood and rumen fluid suggested that rumen acetate levels may be important in this context (Bines and Davey, unpublished results). Infusion experiments by Baile & Mayer (1968) have also shown that rumen acetate is more important than blood acetate in regulation of food intake.

To study this further, a technique is being used in which rumen contents are exchanged between fed and unfed cows receiving a high-concentrate ration. The cows are paired, one of each pair being fed in the morning and the other in the afternoon. Manipulations of rumen contents are made immediately before the afternoon feeding and, in order to assess the effect of these on intake, the cow which received food in the morning is given a second period of access to concentrates in the afternoon. Initially, complete exchanges of rumen contents are being studied but,

since the amount of contents from the fed cow is greater than that from the unfed cow, the amounts are equalized on some days by discarding part of that from the fed cow; other procedures will also be examined.

Some typical results are shown in Table 1.

Table 1. *Effect on intake of concentrates of exchanging rumen contents between fed and unfed cows*

		No. of observations	Voluntary intake* (kg air-dry concentrates)	
			Morning	Afternoon
No rumen emptying:	Fed cow	8	5·84	2·09
	Unfed cow	8	—	7·89
Rumen emptied, own contents returned:	Fed cow	6	6·57	2·31
	Unfed cow	6	—	8·24
Rumen emptied, contents completely exchanged:	Fed cow	3	7·18	7·17
	Unfed cow	3	—	1·02
Rumen emptied, equalized amounts exchanged:	Fed cow	2	5·13	6·63
	Unfed cow	2	—	6·53

*In addition, both cows received 1 kg hay in the morning.

The results show that rumen contents from the unfed cow cause the fed cow to consume an amount of food similar to that which she voluntarily consumed in the morning, whereas contents from the fed cow inhibited intake of food by the unfed cow. The latter effect was reduced, but not completely eliminated, when amounts of rumen contents were equalized, indicating that some constituent of the rumen contents of the fed cow may have had an inhibitory effect on food intake.

REFERENCES

Baile, C. A. & Mayer, J. (1968). *J. Dairy Sci.* **51**, 1490.
Bines, J. A. & Davey, A. W. F. (1970). *Br. J. Nutr.* **24**, 1013.

Further observations on the movements of ammonia in the ruminant and the relationship to ammonia toxicity. By MARGARET I. CHALMERS, I. GRANT, B. THOM and F. WHITE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

It has been shown that ammonia diffuses from the rumen into the peritoneum of sheep and can reach the jugular vein without traversing the liver (Chalmers, Jaffray & White, 1971). Urea or ammonium salts have been administered into the caecum or abomasum of sheep prepared in a similar way, under the same feeding and management conditions, and the movement of ammonia into the vascular system has been studied.

No toxicity was observed when 20 g urea or 10 g of ammonia N were administered into the abomasum: urea or ammonia was absorbed into the mesenteric vein

some hours later. Ammonia toxicity occurred within minutes of administration of urea or ammonium salts into the caecum. Under these conditions the ammonia concentration of jugular blood can be higher than that in the portal vein.

This evidence shows that ammonia toxicity is caused primarily by diffusion of ammonia and that overloading of the liver is not a necessary condition for toxicity.

REFERENCE

Chalmers, M. I., Jaffray, A. E. & White, F. (1971). *Proc. Nutr. Soc.* **30**, 7.