

PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Thirty-sixth meeting of The Nutrition Society will be 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 will be 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 ± 54 , mean CV 19.7; creatinine, mean SD ± 170 , mean CV 16.6.

The overall 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 ± 58 , CV 20; creatinine, mean 1145 mg, range 571-2119 mg, SD ± 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). Results are shown in the table.

Characteristics of oils	Fresh oil		Abused oil		
	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

*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 a 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*

Dietary regimen	Day	Proteolytic activity (% of control \pm SD)	
		Alkaline (pH 9)	Acid (pH 3.8)
Protein-free	21	91.5 \pm 23.2	111 \pm 29.8
	24	129 \pm 15.4**	206 \pm 12.2**
Refeeding	4	44.5 \pm 6.85*	60.5 \pm 4.4*
	7	64.5 \pm 14.5*	62 \pm 10.5*
Starvation	1	100 \pm 12.9	115 \pm 10.9
	2	87.5 \pm 4.97	104 \pm 17.4
	3	107 \pm 25.2	101 \pm 11.9
	4	86 \pm 10.3	88.5 \pm 15.25

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

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 either sucrose or glucose (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 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)*

Day of expt	Group	1		2		3	
		Diet: Stock	SD	Sucrose	SD	Restricted stock	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 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 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 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 ($P < 0.001$) with a time lag and magnitude specific to

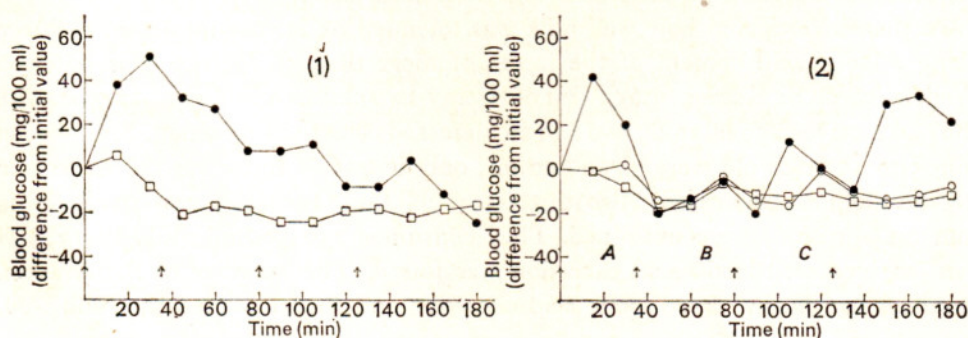


Fig. 1. Mean blood glucose concentration (difference from initial value) of (1) five subjects at rest and (2) five subjects at exercise. \uparrow denotes the taking of a drink; A, B and C are three periods of 4 min maximal gross muscular exercise; \bullet — \bullet , drinking glucose syrup; \square — \square , taking a placebo; \circ — \circ , 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 following 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, *Medical Research Centre, Nairobi, Kenya, Department of the Royal Tropical Institute, Amsterdam* and F. W. M. LUYKEN-KONING, *Central Institute for Nutrition and Food Research TNO, Zeist*

Out of forty-five African children from a boarding-school near Nairobi, twenty-eight were lactose-intolerant, i.e. their blood glucose increased by less than 20 mg/100 ml after oral loading with 2 g lactose/kg body-weight. 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 are shown in the table.

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 ($\mu\text{mol/ml}$) 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 fed 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 Tremolières & 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 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 experiments, 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 & 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 ($P \leq 0.05$) 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

*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.

KRB 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, Teeban & 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. Three groups each of eight Sprague-Dawley male rats at weaning 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 fed 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.

KRB 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 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 fed diets containing starch or sucrose*

Dietary group	Plasma		Fatty acid synthetase (units/g liver)	Lipoprotein lipase (units/mg N)
	Triglyceride (mg/100 ml)	Cholesterol (mg/100 ml)		
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 smaller increase in triglyceride level evoked by dietary sucrose in female animals cannot be explained by differences in the activity of these enzymes.

KRB 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). There has consequently 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 22 (13 male, 9 female); no change (3 male, 6 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

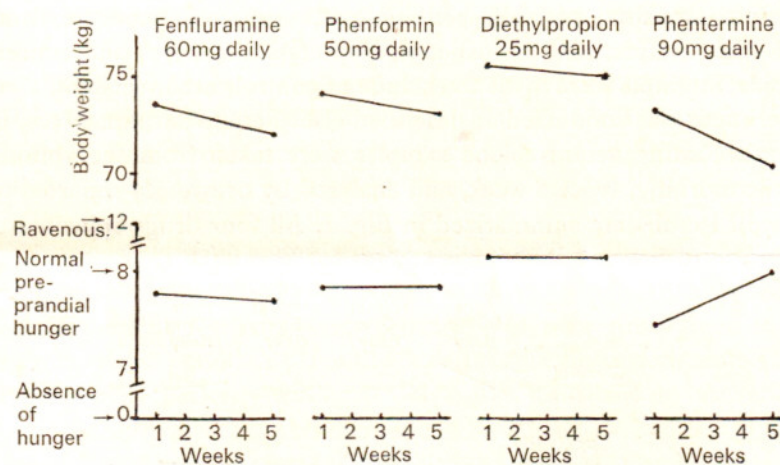


Fig. 1. Effects of anorexic 'antiobesity' agents on body-weight and 'linear appetite rating' in man (mean results)

part of the trial. There were 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 effect

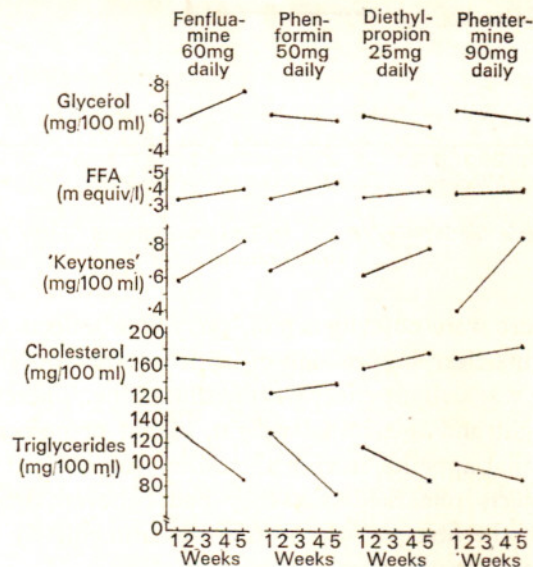


Fig. 1. Effects of 'antiobesity' agents on blood plasma lipids in man (mean values).

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, while 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 (1968a) 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, 1968b).

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, whilst 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- and low-protein 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	117	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 following weaning in strains of mice selected for mature body-weight. By MARGARET W. STANIER and L. E. MOUNT, *ARC 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. During suckling 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

following 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 the case 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 following 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*
NDP/Cal%	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/1000 kcal)	0.40	0.47	0.37	0.55*	0.45	0.57*
Vitamin B ₂ (mg/1000 kcal)	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 (μ g)	390	2230*	530	2110*	1050	1320
Vitamin D (μ g)	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		Prescribed daily intake	Total intake	Intake from hospital supplies
Specified reducing diets	Total days			
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

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 which were given barley-based diets until they were slaughtered when 4 months old, and, for comparison, to two year-old lambs 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. †Including anteiso acids (mostly 17:br) and several unidentified.

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 6 to 4 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	46	5	10	8
5 }	2	21	1	16	40	4	7	5
6 }	3	20	1	12	45	5	8	7
7 } Butyrate diet	2	23	3	17	42	3	3	2
8 }	3	27	3	17	41	3	3	2
9 }	3	24	3	17	41	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.

†Including *anteiso* acids (mostly 17:br) and several unidentified components.

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.

Whilst 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, *ARC 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.

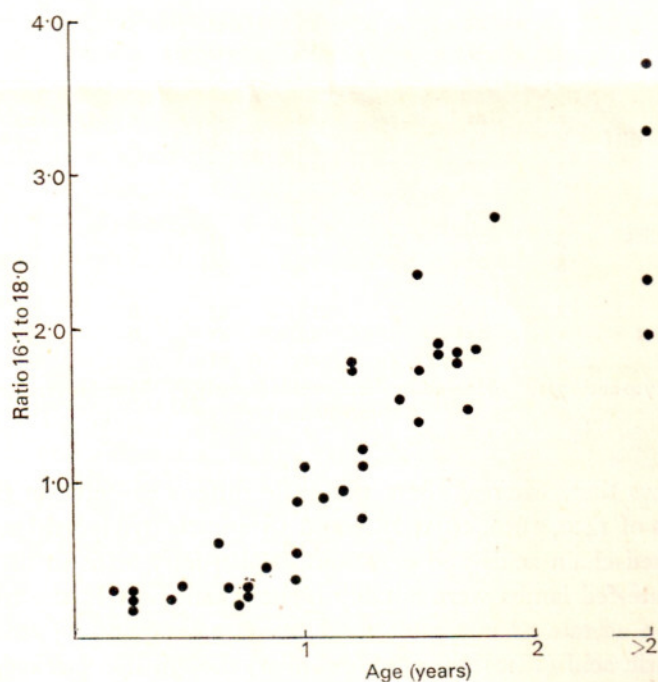


Fig. 1. Variation with age in the ratio of $C_{16:1}$ to $C_{18:0}$ in subcutaneous adipose tissue of Jersey cattle.

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} to 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 fed 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 on 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 (ME) 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 NH₃ 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 NH₃ 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. ByA. 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 \pm 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 stalled 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, 1971a,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)

Treatment	Crude protein		Non-ammonia crude protein		Crude protein	
	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 and 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 was 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 was insufficient to meet the requirement of the rumen micro-organisms.

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The effect of urea supplementation of 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, molasses 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:

$$\text{Abomasal N flow} = 0.48 (\text{dietary N ingested}) + 7.8,$$

$$\text{Abomasal N flow} = 0.32 (\text{N ingested} + \text{N recycled}) + 7.7.$$

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 while 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 cannula, 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 2M-H₂SO₄ for 4 h at 100°. Amounts of sugars liberated from the bacteria (g/100 g dry matter, mean values \pm standard error), estimated by ion-exchange chromatography of their borate complexes (Smith & McAllan, 1971), were xylose (0.28 \pm 0.05), arabinose (0.29 \pm 0.10), rhamnose (1.00 \pm 0.26), ribose (0.70 \pm 0.09), mannose (0.42 \pm 0.06), galactose (2.74 \pm 0.86) and glucose (15.46 \pm 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 \pm 0.62); this was still lower (1.56 \pm 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 \pm standard errors) were xylose (7.06 \pm 0.94), arabinose (2.04 \pm 0.25), rhamnose (0.46 \pm 0.07), ribose (0.27 \pm 0.04), mannose (0.17 \pm 0.02), galactose (1.69 \pm 0.21), 'starch'-glucose (9.42 \pm 1.34) and 'cellulose'-glucose (10.78 \pm 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.

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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 \pm 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 the values reported above 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 (\pm standard error) 'cellulose'-glucose digestibility was $1 \pm 4\%$. 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 $64 \pm 3\%$ 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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