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ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Sixty-third Scientific Meeting of the Nutrition Society was held in Guy's Hospital Medical School, St Thomas Street, London SE1 9RT, on Friday, 7 December 1973, *at* 10.30 *hours, when the following papers were read :*

Effect of feeding with sucrose and other carbohydrates on platelet function in rats. By LILIAN MCGREGOR, *Queen Elizabeth College, London W8 7AH*

There is very little information on the effect of high-carbohydrate diets on platelet function in rats and what there is describes the effects of glucose on platelet aggregation (Davis, Wilson & McField, 1967).

The purpose of this study was to compare the effects of sucrose-, starch- and glucose-containing diets on maximum rate of shape change (V_{Smax}, arbitrary units/min per 10^8 platelets) (Michal & Born, 1971) and on the maximum rate of aggregation (V_{Amax}, transmission units/min per 10^8 platelets) (Born, 1962) of rat platelets in vitro using ADP as the aggregating agent.

The first experiment continued for 120 d. Nineteen male litter-mate Sprague-Dawley rats were fed on diets containing 690 g of sucrose, starch or glucose per kg. The diets contained (g/kg): maize oil 20, casein 230, minerals 40, and vitamins 20. V_{Smax} was significantly faster in rats given starch.

Table 1. *Effect, in rats, on body-weight gain, number of platelets, V_{Smax}, and V_{Amax} of diets containing sucrose, starch or glucose*
(Median values with ranges in parentheses)

Diet	Wt gain (g)	No. of platelets (10^8/ml)	V_{Smax} (arbitrary units/min per 10^8 platelets) (2 μmol ADP†)	V_{Amax} (transmission units/min per 10^8 platelets) (2 μmol ADP)
Sucrose	161 (140–188)	9·2 (8·1–12·2)	240 (194–334)	165 (121–212)
Starch	171 (140–195)	8·9 (6·9–10·8)	320 (270–390)*	166 (138–212)
Glucose	178 (150–185)	9·2 (7·3–11·1)	232 (184–382)	150 (111–182)

*$P<0.05$.
†, Aggregating agent.

A second experiment was carried out in the same way with diets containing sucrose or starch, but the diets were given for a shorter period (18 d) and both thrombin and ADP were used as aggregating agents. In this instance there were no significant differences in V_{Smax} or V_{Amax}.

In a third experiment, adult male rats were meal-fed for 4 h every day (11.00 to 15.00 hours). Diets contained 690 g sucrose or maize starch per kg, and otherwise

resembled those described above. There were seventeen rats in each group and the experiment continued for 84 d. There were no significant differences in platelet behaviour.

These results recall the findings of Hornstra (1971) who found more rapid thrombosis of aortic loops in rats given starch compared with those given sucrose.

I am grateful to Professors A. S. Truswell and J. Yudkin and to Dr R. Bruckdorfer for their help.

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Dental plaque formation in relation to type of carbohydrate. By T. H. GRENBY and G. EIKREM, *Department of Oral Medicine and Pathology, Guy's Hospital, London SE1 9RT*

Recent experimental work has presupposed an association between dental caries and the accumulation of dental plaque, but firm evidence has been lacking. Although it is known that the carbohydrate content of the diet can influence the composition of the plaque, little information exists on the relationship between plaque deposition and the form in which the carbohydrate is ingested.

These points were investigated in a survey of the dental condition and eating habits of a group of dental students, and the effect on the plaque of replacing some of the dietary sucrose by a low-energy sweetener was tested.

Twenty-four students were examined clinically, and individual DFS scores (measurements of decayed and filled tooth surfaces) were compiled. Each student completed a questionnaire on sucrose intake.

The students' teeth were scaled and polished at the start of a 3 d period on their normal diet, during which they did not brush their teeth. The plaque which formed was stained and photographed. The area of plaque was scored on a 0–5 scale, and a mean plaque score per tooth (PST) was calculated for each subject.

In the test of the low-energy sweetener the same procedure was followed, but the subjects were asked to substitute it for sucrose whenever possible during the 3 d period. The low-energy sweetener had half the bulk density of sucrose, and contained 98% glucose and its α-1,4-linked oligomers, plus saccharin to give it the same sweetness by volume as sucrose.

A significant positive correlation ($P<0{\cdot}05$) was established between the individuals' DFS scores (caries experience) and plaque formed in 3 d on the normal diet (PST). There was a significant positive correlation ($P<0{\cdot}01$) between the mean PST and the subjects' stated daily consumption of sugar in tea and coffee. When the PST after the two 3 d periods were compared, the scores were significantly

lower ($P<0.05$) on the low-energy sweetener (mean 2.27 ± 0.13) than on the normal diet (mean 2.55 ± 0.09).

These findings show that the extent of dental plaque increases with sucrose intake from sweetened drinks. The subjects who developed the most plaque were those who had the highest caries experience. The reduction in plaque after replacing sucrose, mainly in drinks, by a glucose-based sweetener, suggests a possible long-term measure for improving dental health.

Our thanks are due to the twenty-four student volunteers, and to Tenstar Products Ltd for the supply of the sweetener.

The differing response shown by male and female rats given low-protein—high-carbohydrate diets. By D. B. JEFFERYS and I. R. WHITE, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

The female rat appears to be more resistant to the effects of protein depletion than the male (Radhakrishnan, 1966). Stephen (1968) fed young male and female rats on low-protein—high-sucrose diets and reported that the female showed more adaptation to the regimen.

We found that female sex hormones can affect the response of male rats to sucrose (Jefferys & White, 1973*a*) and that the nature of the carbohydrate (starch or sucrose) given to male rats also influences the change in serum protein concentrations when these rats are given a low-protein—high-carbohydrate diet (Jefferys & White, 1973*b*). It was therefore decided to investigate whether the nature of the carbohydrate in the diet influenced the difference in response in serum protein concentrations seen between male and female animals.

Three groups of male Wistar rats (290 g) and three groups of females (240 g) were used, five rats/group. Male and female control groups received (g/kg food): protein 150, carbohydrate 660, fat 130, salts 10 (Jefferys & White, 1973*b*) (18·4 MJ/kg). The other groups received (g/kg food): carbohydrate (sucrose or starch) 800, fat 130, protein (calcium caseinate) 50 and salts 20 (18·0 MJ/kg). All the food was offered *ad lib.*

After 27 d, the rats were fasted for 12 h and blood was removed from the heart under heavy diethyl ether anaesthesia. The concentrations of serum proteins, serum lipids and liver lipids were then determined (Jefferys & White, 1973*b*).

There was no significant difference in food consumption between the male and female groups when allowance was made for body-weight. The female groups, however, showed less weight change than the males.

No difference was found between the sexes in the total serum protein values for the control groups. The low-protein diets significantly decreased the serum albumin concentration in all the experimental groups when compared with the controls ($P<0.001$). The albumin concentrations were higher in the female than in the male

groups ($P<0.01$). The males receiving sucrose had a higher albumin level than those given starch, but no such difference was found for the female animals. The total serum lipids were increased in all the experimental animals on the low-protein diets. The serum lipid concentration for the male rats given sucrose was markedly raised. The total liver lipid concentrations were significantly raised for the sucrose and starch groups in the male, but not in the female.

This experiment confirmed that the female rat is more resistant to the effects of protein deficiency than is the male. The nature of the carbohydrate in the diet affects the nutritional status of the male but not of the female. Therefore, from the results of these experiments, it would seem that the type of carbohydrate in low-protein diets does not account for the difference in protein response between the sexes.

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The influence of fat in the meal on oral glucose tolerance. By A. R. MACRAE and I. MACDONALD, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

It has been demonstrated recently that the addition of fat to a glucose meal results in an impairment of the glucose tolerance in man (Taylor, Macdonald & Henderson, 1971). It was decided to investigate further the cause of the impaired glucose tolerance by comparing an emulsified fat (cream) with an unemulsified fat (sunflower-seed oil).

Following an overnight fast, healthy men and women (mean age 23 years) were given test meals that contained either glucose alone (1 g/kg body-weight), or the same dose of glucose together with fat (1 ml/kg body-weight). All test meals were made up with water to a total volume of 4 ml/kg body-weight.

In one experiment with sunflower-seed oil, the subjects were lying prone on the left side; in all other experiments with both types of fat the subjects were in a sitting position. In another experiment, the amount of cream fat in the meal was varied from 0·4 to 1·5 ml/kg body-weight, while maintaining the same total volume of test meal. Capillary or venous blood samples were taken before the meal and at 15 min intervals up to 1 h after it. Blood glucose was estimated by an automated glucose oxidase method (Faulkner, 1965).

The peak concentration of glucose in the blood after the test meal was reduced if cream fat was present in the meal. The greater the amount of cream fat added, the less was the subsequent rise in blood glucose. With sunflower-seed oil added to the glucose meal, the effect was determined by the position of the subject. In the prone position, when fat could enter the duodenum by flotation, there was practically

no rise in blood glucose; in the sitting position, on the other hand, the rise in blood glucose was significantly greater than with glucose alone.

These results are consistent with the view that the variations in the glucose tolerance previously reported are in all probability due to the degree of separation of the fat from the water phase while in the stomach.

We are grateful to the volunteers. This study was supported by a grant from the Wellcome Trust.

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The influence of feeding pattern on rat growth and development. By R. C. POCKNEE and F. W. HEATON, *Department of Biological Sciences, University of Lancaster, Lancaster LA1 4YQ*

Many dietary deficiency studies involve the pair-feeding of animals on a daily basis, but although this equates the total food intake, it often leads to the development of different feeding patterns. With rats, control animals adopt a meal-eating pattern of feeding, whereas deficient animals consume food at frequent intervals throughout the 24 h period.

Two groups of weanling male Wistar rats received a synthetic diet of normal composition for 24 d. Meal-eating rats were allowed access to food for 2 h each morning, and controls received an amount of food equal to that consumed by the experimental animals, but were fed automatically (Loveless, Williams & Heaton, 1972) to ensure a normal frequency of food intake throughout the day. On the final day each group received half the normal amount of food and the animals were fasted overnight before killing.

The gain in total body-weight was similar in animals of both groups, indicating a similar efficiency of food utilization. The fresh weights of the liver, kidneys, femur, small intestine and stomach were, however, greater ($P<0{\cdot}01$) by 10–23% in meal-eating rats than in control animals. No significant differences were observed in the large intestine, testes, heart or submaxillary glands, and the spleen was the only organ found to decrease, although the lighter weight of the remaining carcass in experimental animals indicated that other tissues were also reduced in size.

Chemical analysis of organs revealed no difference between the water, nitrogen or lipid concentrations in liver, kidney or spleen in the two groups of rats: the proportions of water and ash in the femur were also unaltered. The ratio dry matter:DNA was increased in the kidney of meal-eating rats compared with control animals ($P<0{\cdot}001$), indicating that the hypertrophy of this organ was associated with an increase in cell size, but no differences were found in liver or spleen.

Hypertrophy of the gut in meal-eating animals is well established (Fábry, 1969), but our investigation indicates that feeding pattern has more widespread effects on

the body and influences the development of organs not directly concerned with the intake of food. As no differences in tissue composition were observed, this appears to be the result of an effect on growth rather than on the metabolism of one type of cell constituent.

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The effects of caffeine and carnitine on the oxygen consumption of fed and fasted subjects. By D. S. MILLER, M. J. STOCK and JEAN A. STUART, *Departments of Nutrition and Physiology, Queen Elizabeth College, London W8 7AH*

Part of the study on the role of dietary-induced thermogenesis in energy balance being carried out at this college is concerned with assessing the ability of various agents to potentiate the normal thermic responses produced by feeding. The present paper reports on the effects of caffeine and carnitine on the oxygen consumption of fed and fasting subjects.

Fasting subjects (three men and three women) were given, on four separate occasions, 250 mg caffeine, 250 mg each of caffeine and carnitine, a 2·93 MJ (700 kcal) breakfast, and finally, all three in combination. Oxygen consumption was measured as previously described (Stock, Stock & Stuart, 1973).

A summary of the results for the six subjects is shown in Table 1. It can be seen that caffeine, given in a dose approximately equivalent to two cups of coffee,

Table 1. *Mean oxygen consumption (ml/min, $\dot{V}O_2$) of the subjects*

	Caffeine	Caffeine + carnitine	Breakfast	Breakfast + caffeine + carnitine
Preprandial	225	227	230	229
Postprandial	256	255	266	287
	$P<0·05$	$P<0·05$	$P<0·05$	$P<0·05$
$\Delta \dot{V}O_2$	31	28	36	58

has a marked effect on oxygen consumption, equivalent to the thermic effect of the breakfast. The mode of action of caffeine is not known, but if it were related to its lipolytic effect (due to inhibition of phosphodiesterase) one might expect carnitine to enhance the increase in oxygen consumption, as has been shown for the thermogenic effects of noradrenaline (Hahn, Skala & Davies, 1971). However, it will be noticed that a combination of the two compounds produced an increase that was not significantly different from that of caffeine alone. Giving both drugs with the breakfast produced an increase significantly greater than that of any of the other

treatments ($P<0.05$). The increase is equivalent to one-quarter of the fasting metabolic rate.

It would appear that caffeine, by virtue of its negligible energy value and thermogenic properties, could be of value in promoting the loss of body energy on slimming regimens.

We thank all those who volunteered as subjects.

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Dental splinting in the treatment of hyperphagic obesity. By J. S. GARROW, *Clinical Research Centre, Watford Road, Harrow*

Although it is not generally true that obese patients eat excessively, there is a recognizable group of grossly obese patients who describe themselves as 'compulsive eaters'. Characteristically they go through cycles of concern about their disabling obesity—a period of dieting lasting weeks or even months with good weight loss, a sudden collapse in their will to diet often associated with some emotional crisis, a period of massive overeating with consequent rapid weight gain—and thus reach the beginning of the cycle once more. Dietary advice in such cases is ineffective, and in-patient treatment on a low-energy diet produces weight loss, but does nothing to assist the patient to resist the temptation to overeat when he again is faced with domestic crises after discharge from hospital. Attempts have been made to treat these patients by surgical bypass of the ileum, or by destruction of the hypothalamic feeding centres. These radical forms of treatment have been only moderately successful, and they carry a considerable risk of doing more harm than good.

It seems that the best chance of breaking the vicious circle is at the point when some upset precipitates an eating binge. The patients themselves have no forewarning of this, nor are they able to stop eating although they know it is not in their interests to eat. We have therefore referred some patients to Dr G. L. Fordyce who has fitted dental splints of the type used in the treatment of fractured jaws. These have proved acceptable to the patients, who can now drink but not eat. Their appearance and speech is little affected, and they have continued at work, or looking after their families. When the inevitable crises have occurred, to which the patients would formerly have responded by overeating, they have found some alternative response. The two patients who have had the splints removed after a period of about 5 months have each lost more than 40 kg, and at that stage were confident that they had learned to control their impulse to overeat. Follow-up results will be reported.

Changes in body size relative to age and to childbearing in Papua New Guinea women: a comparison of Highlands women and coastal women. By HEATHER GREENFIELD and JANE CLARK, *Institute of Medical Research, Papua New Guinea*, and IAN RING, *University of Papua New Guinea, Port Moresby*

A study of Highlands women living on diets of sweet potato low in energy and protein showed that, although there was a progressive loss of body-weight with age, there was no correlation with repeated cycles of pregnancy and lactation. The exception was the first cycle, which was associated with loss of body-weight and fat, as shown by skinfold measures at various sites (Greenfield, Clark & Serjeantson, 1973).

Coastal women living on a higher nutritional plane (tuber and fish diets) have been studied for comparison in (1) remote off-shore islands of New Ireland and (2) villages near Kavieng, the main town of New Ireland. Dietary and anthropometric results will be presented for comparison with those obtained from the Highlands women.

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The measurement of portal and hepatic blood flow in pigs. By D. M. ANDERSON, *Department of Biochemistry, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

Pigs were prepared with indwelling catheters in the portal vein, hepatic vein, right atrium and aorta. Blood flow was measured simultaneously in the portal and hepatic veins by the *p*-aminohippuric acid (PAH) infusion method of Katz & Bergman (1969) and at the same time hepatic vein flow was measured by the bromsulphonphthalein (BSP) method of Bradley, Inglefinger, Bradley & Curry (1945).

The BSP method was repeatable both within and between experiments in the same animal and in pigs (five) weighing $27 \pm 1{\cdot}6$ kg, hepatic blood flow was 1810 ± 70 ml/min (thirty-one observations) after 18 h without food, and 1950 ± 60 ml/min (ninety-eight observations) during the 4 h after feeding.

The estimates of hepatic vein blood flow made by the PAH method were extremely variable both within and between animals and, very frequently, flow-rates which exceeded the probable cardiac output were obtained. There was also poor agreement between PAH and BSP estimates. It could be shown that the liver removed or denatured a small quantity of PAH and that this was not due to acetylation of PAH. However, this loss was not large enough to account for the high PAH flow values. When a catheter was established in both the major hepatic veins, there were differences in PAH concentration between the two veins when PAH was infused into the portal vein but not when it was infused into the right atrium. This would indicate that the differences were due to incomplete mixing of PAH in the portal vein and not due to differential uptake by the lobes of the liver. Further

evidence of incomplete mixing of PAH in the portal circulation was adduced from the fact that, in some animals, obviously aberrant values for portal flow reverted to more acceptable values when the animal was fed, and also, on some occasions portal blood flow was greater than hepatic, while both showed a pattern similar to that of BSP blood flows. Infusion of PAH into the portal vein through up to three catheters had no beneficial effect on mixing. These results would suggest that in the pig any method of determining portal blood flow which depends upon the infusion of known amounts of a substance into a portal radical and the measurement of its dilution must be suspect, no matter how large the volume infused.

Where there were no obvious signs of incomplete mixing of PAH in the portal vein, portal blood flow was in the region of 40–60 ml/kg body-weight per min which, along with the BSP hepatic vein flows, indicates that portal vein and hepatic vein blood flows in the pig are higher than those reported for other species.

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Measurement of portal blood flow in the pig by the continuous thermal dilution technique. By F. WHITE, A. J. F. WEBSTER, D. J. FARRELL* and A. S. JONES, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The adaptation of the continuous thermal dilution technique for measurement of portal blood flow (Webster & White, 1973) in conscious sheep has been successfully achieved in the conscious pig. The surgical procedures followed and the equipment used to measure blood flow were similar to those described by Webster & White (1973).

The branches of the anterior mesenteric vein from the small intestine are usually much more numerous and smaller in the pig than in sheep; this made implantation of the infusion catheter and the double-bore catheter holding the copper–constantan thermocouple more difficult. However, the portal vein of the pig is considerably longer than that of the sheep, and correct placement of the thermocouple in the vein was more readily achieved under X-radiography with image intensification.

The lowest blood flow, of 1·1 l/min, was observed in two pigs that had been starved for 48 h. The highest value, of about 3 l/min, was observed in both pigs 2–3 h after feeding. Our range of values is similar to that reported by Braude, Cutts, Myres & Porter (1970), who used a dilution method with ^{131}I as the indicator to measure blood flow in the portal vein of a 35 kg pig. Rerat's (1973) values obtained using an electromagnetic technique were usually lower than the readings we observed in pigs in the same live-weight range during the 8 h period after feeding.

The increase in portal blood flow of two pigs for the 8 h after feeding is shown

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Table 1. *Increase in portal blood flow of two pigs expressed as a percentage of the mean of the prefeeding values, observed during each hour during the 8 h after feeding*

(Values in parentheses are the standard errors of the mean)

Pig no.	1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
1	41 (7)	74 (9)	56 (13)	35 (9)	28 (3)	29 (5)	37 (7)	26 (9)
2	37 (7)	61 (9)	44 (6)	46 (8)	54 (24)	42 (10)	41 (18)	38 (7)

in Table 1. Values observed during each hour were grouped into hourly readings and are expressed as a percentage increase of the mean of two to four measurements made during the 90 min period immediately before feeding. Pig no. 1 was offered daily a ration of 1600 g of a barley-based standard diet, and pig no. 2, 1100 g. The number of observations for each pig was in excess of 50, recorded on 6 d. The peak value in flow rate was observed for both pigs during the 2nd hour after feeding and a second, but smaller peak, was seen some hours later.

Pig no. 2 was killed while in good health after 62 d because the infusion catheter became blocked. In pig no. 1 excellent measurements were obtained for 103 d until the thermocouple wire broke internally. During this time pig no. 1 doubled its body-weight and on the day before slaughter consumed 2·5 kg food.

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Blood flow and free amino nitrogen (FAN) concentration in the portal vein of the pig. By D. J. FARRELL*, A. S. JONES, A. J. F. WEBSTER and F. WHITE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The difference between the concentration of amino acids in whole arterial and portal blood, adjusted for differences in rates of flow, is a measure of the absorption of amino acids from the gut. It has been suggested, however, (Buraczewska, Tas, Axford, Evans & Chamberlain, 1972) that erythrocytes may play a significant part in the transport of amino acids, and experiments have therefore been done to estimate the relative importance of plasma and erythrocyte transport. Samples of plasma and whole blood from portal veins of two pigs were analysed for FAN both before and after feeding. Portal blood flow was estimated by the technique of Webster & White (1973). Shown in Fig. 1 is the very rapid increase in blood flow of two pigs that were fed after 48 h starvation on 2 consecutive days. After the first measurement there appeared to be a transitory increase in flow-rate before feeding; this probably reflected anticipation of receiving the daily ration of 1500

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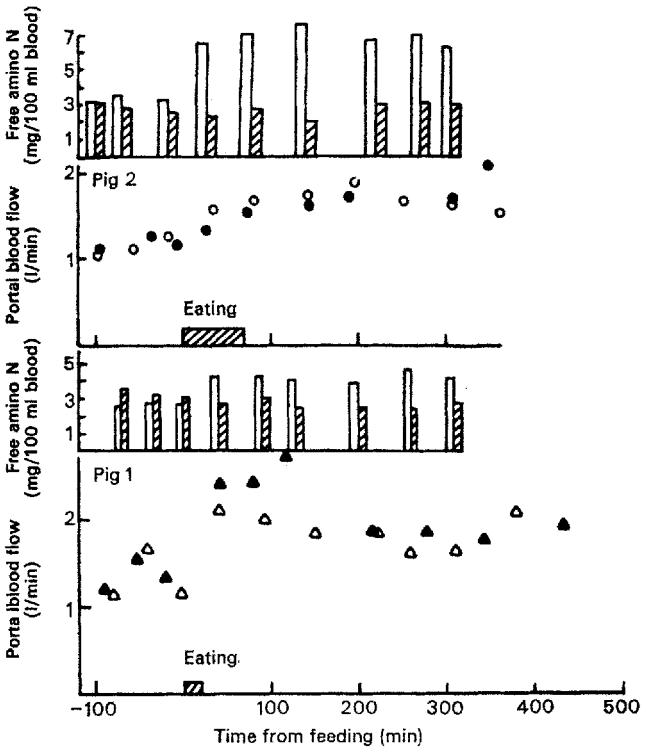

Fig. 1. Portal blood flow for pig 1 (67 kg) measured on day 1 (▲) and day 2 (△), and for pig 2 (45 kg) measured on day 1 (●) and day 2 (○). Concentration of FAN in plasma (□), and erythrocytes (▨), measured on day 2, is shown for each pig.

and 1100 g of a barley-based diet offered to pig nos 1 (67 kg) and 2 (45 kg) respectively, and has also been observed in other experiments.

The concentration of FAN, measured only on the 2nd day, showed a more marked increase in the plasma portion of whole blood (mg/100 ml) than in erythrocytes following feeding of pigs. Although pig no. 2 consumed a smaller amount of food over a longer period of time than pig no. 1, there was a much larger increase in FAN concentration of samples taken from pig no. 2.

Although plasma FAN appears to be a more sensitive index of changes that occur in the FAN concentration of whole blood, the observation that there are differences in the rate of change of FAN concentration in plasma and erythrocytes suggests that for the quantitative measurement of amino acid absorption from the digestive tract, whole-blood samples should be analysed.

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

In order to use changes in concentrations of plasma amino acids (PAA) and plasma urea nitrogen (PUN) in calves to assess amino acid requirements, it is necessary to have information on background variations in these measurements. Such variations have been studied in 110–160 kg Friesian calves given equal daily amounts of different concentrate mixtures and straw, the former at 10.00 and 17.00 hours, the latter at 17.00 hours only. All the diets provided energy intakes for a growth rate of 0·4 kg/d and any one was given for 3 weeks before sampling.

Two calves were given a diet (A), containing 20 g nitrogen/kg dry matter, in which the concentrate mixture was flaked maize, starch, glucose and decorticated groundnut meal (DCGM). Samples of jugular blood were taken at hourly intervals for 24 h. Total PAA increased by about 27% 1 h after the morning feed, but returned to near the prefeeding level 2 h later. This level was maintained in further samples taken before the afternoon feed. Changes after the afternoon feed were rather irregular and there was no clear indication of the increase shown after the morning feed. Individual amino acids and PUN showed similar patterns of change. Samples in subsequent experiments were taken 3 h after the morning feed. Such samples from ten calves, all given diet A, showed very wide variations between animals, much greater than the variations found within animals over periods of up to 7 weeks (Table 1).

Table 1. *Plasma composition of calves given diet A (see above)*

(Mean values with their standard errors)

No. of samples	10	7 (at weekly intervals)				5 (at daily intervals)	
No. of calves	10	1	1	1	1	1	1
Plasma amino acids (μmol/l)	1925±121	1820±41	1754±53	2387±68	1411±37	2291±54	2060±25
Plasma urea nitrogen (mg/l)	62±8	98±3	39±2	55±3	65±3	61±3	44±3

Effects of varying the amounts and types of protein supplements in the diet were studied by giving individual animals different diets in alternate periods. Replacing the DCGM in diet A with an isonitrogenous amount of maize gluten (diet B) led to PUN nearly doubling. There was little change in total PAA and the only individual amino acids to show appreciable changes were arginine, which was halved, and leucine, which doubled. These changes apparently reflected differences in dietary composition despite the masking effect of microbial protein synthesis. Replacing part of the DCGM in diet A with starch and glucose to give 10 g nitrogen/kg dry matter (diet C) led to PUN being nearly halved, but total PAA did not appear to change greatly. Of the individual amino acids, isoleucine, alanine and arginine

apparently decreased by 25%, while glutamic acid and glycine increased by similar amounts.

The amino acid requirements of the ruminating calf. By A. P. WILLIAMS and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

A number of estimates of the amino acid requirements of sheep have been made (e.g. Wakeling, Lewis & Annison, 1970), but there is no comparable information for the growing calf. Experiments have been carried out with calves receiving the diets described by Williams & Smith (1974). Six Friesian calves (110–160 kg), fistulated in the abomasum, received diet A containing 20 g nitrogen/kg dry matter. Chromic oxide-impregnated paper was given twice daily with the feeds as a marker and daily flows of amino acids at the abomasum were estimated from the composition of abomasal digesta samples. Varying amounts of L-methionine were infused, for periods of 4 d, into the abomasum of the calves. Plasma methionine concentrations showed little response at the lowest infusion levels, but rose markedly when higher levels were infused (Fig. 1). Linear regression lines expressing these different types of response intersected at a methionine infusion rate of 4·6 g/d. Evidence from other species indicates that this corresponds to the point at which the methionine requirements were met (e.g. Zimmerman & Scott, 1965). Estimated flows of methionine and cystine passing into the abomasum from the rumen (mean values

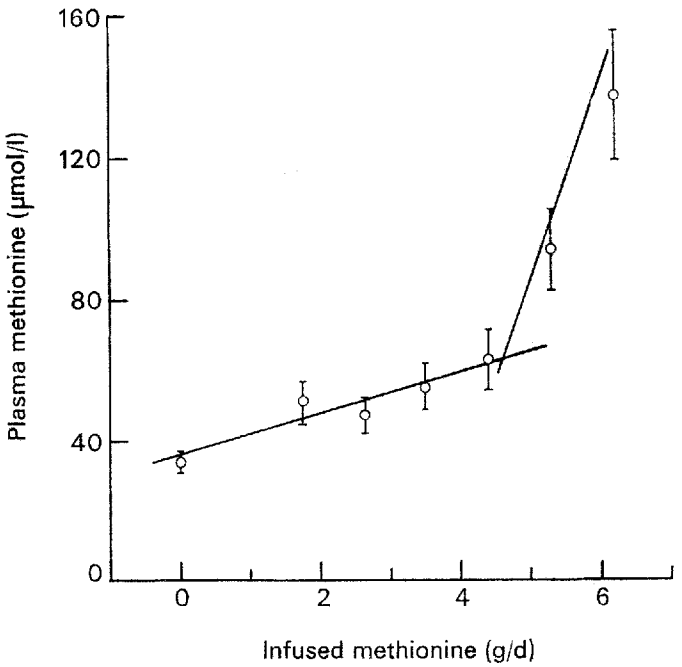

Fig. 1. Effect of abomasal infusion of methionine on plasma methionine concentrations of the calf.

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with their standard errors for four calves) were $5{\cdot}4 \pm 0{\cdot}3$ and $4{\cdot}9 \pm 0{\cdot}3$ g/d respectively. It appeared, therefore, that with this cystine supply, methionine requirements for these calves was 10·0 g/d. In other experiments, for two calves given diet C containing 10 g nitrogen/kg dry matter, estimated methionine requirements appeared to be lower (7·5 g/d). Similar experiments were carried out, with maize gluten providing the dietary protein supplement to give 18·8 g nitrogen/kg dry matter (diet B) and with infusion of L-lysine into the abomasum. Plasma lysine increased linearly with increasing levels infused, suggesting that the calves' requirement for lysine was less than the 20 g/d at the duodenum provided by this diet. Neither methionine nor lysine infusions had any marked effect on plasma urea nitrogen concentrations.

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On the new interpretation of recommended intake for protein. By P. V. SUKHATME, *Gokhale Institute of Politics and Economics, Poona 4, India*

A new interpretation has recently been placed on the meaning of recommended intake for protein, in evaluating intake values (FAO/WHO, 1973) namely that 'as the intake falls below the recommended level, the risk of dietary deficiency increases'. The validity of this interpretation and its implications are examined in the paper.

Previously, protein requirements were defined at three levels, $m+2\sigma$, m, and $m-2\sigma$, with m denoting the average and σ the standard deviation of individual requirements of the specified age–sex group (FAO/WHO, 1965). Of these, $m+2\sigma$ is called the recommended intake, and is 'expected to cover the needs of all but a very small proportion of the population'. The lower level, $m-2\sigma$, represents the level 'below which protein deficiency may be expected to occur in all but a small proportion of the population'. The coefficient of variation was then estimated at 10%.

In contrast to this, the FAO/WHO (1973) report on protein and energy requirements refers only to the upper level, $m+2\sigma$, which it defines as a 'safe' level of intake. The estimate of σ is revised upwards to 15% of the average requirement m; at the same time the meaning of σ is elaborated. It is stated that part of the variation will be between individuals (σ_b), and part will be between periods within individuals (σ_w), but most of the variation in the Committee's view will be biological arising from differences between individuals, i.e. σ_w is assumed to be zero.

Values available in the literature have been examined to see how far they support the Committee's viewpoint. It is found that far from being negligible, σ_w often accounts for the major part of the total variation.

This paper presents a mathematical model to study the implications of this finding. In the model the deviation of individual requirement from the average value is

expressed as the sum of two independent random variables, one reflecting inter- and the other intra-individual variation of requirement. Expressions are derived for calculating the probable upper and lower limits to the incidence of protein deficiency in a population. It is shown that the incidence will be grossly exaggerated if σ_w is assumed to be zero and σ_b is taken as 15% of the mean value.

The 1973 report explicitly states that an individual eating below $m+2\sigma$ will not necessarily be malnourished; nevertheless it is stated that it is desirable to limit the proportion of such people in the population to 2·5% or to even a smaller percentage. The implication of this suggestion in relation to the need for increasing available protein supplies has been examined by Larstadt (1971) and by Beaton (1972) and their analysis will be re-appraised.

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A simple technique for the estimation of tissue protein turnover in man.

By D. HALLIDAY, J. S. GARROW and P. A. RODGERS, *Clinical Research Centre, Watford Road, Harrow*

The measurement of the absolute rate of total body protein turnover in man is very difficult (Waterlow, 1969). If an isotopically labelled amino acid is injected into the metabolic precursor pool, and if the rate of accumulation of the isotope in protein is known, then the rate of protein synthesis can be calculated provided that the specific activity of the precursor, at the point where the protein is being made, is also known. This last requirement is virtually impossible to meet by any technique which can ethically be applied to man. We have therefore adopted a technique which, although it cannot be shown to give a valid measurement of absolute protein turnover, should permit comparison of tissue protein turnover rate in individuals with the same energy and protein intake. The technique involves a single dose of [^{15}N]glycine, and complete collection of urine for the next 2 weeks. The first 5 d after the dose is disregarded for calculation of the turnover rate, and the turnover is calculated from the daily excretion of ^{15}N from day 6 to day 15, expressed as a percentage of the dose remaining at the beginning of each day.

The immediate application of this technique is in the investigation of the metabolic adaptations of obese patients when they are put on a low-energy diet. It has been amply demonstrated (Bray, 1969) that there is a marked decrease in resting metabolic rate in obese patients under treatment, which implies either that the normal metabolic processes continue at a lower cost in oxygen uptake, or alternatively that there is a reduction in the amount of metabolic work which is done. Reasons will be given for considering the latter explanation to be the more probable and, of the possible savings in resting metabolic work, the most likely is by a reduction in the rate of

tissue protein turnover. Preliminary results in support of this hypothesis will be presented.

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Insulin and growth hormone secretion in the newborn offspring of rabbits fed from mating on high-protein or low-protein diets. By M. R. TURNER, K. A. ALLEN and K. A. MUNDAY, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

The secretion of insulin from isolated pancreas in vitro in response to glucose and to amino acids, and the secretion of growth hormone (GH) in vivo in response to an intraperitoneal arginine challenge, have been measured in the newborn offspring of New Zealand White rabbits, reared on a commercial rabbit diet and fed from mating on diets containing either 200 or 100 g soya-bean protein/kg.

The isolated pancreas from offspring of rabbits fed on the high-protein diet (control offspring) did not respond in vitro to glucose stimulation (16·5 mmol/l) nor to arginine (5 mmol/l) but there was a significant insulin secretion when leucine was included in the medium (5 mmol/l). On the other hand, the pancreas from offspring of rabbits fed on the low-protein diet (LP offspring) responded to both glucose and leucine, but not to arginine. The insulin responses both to glucose and to leucine were significantly greater in LP offspring than in control offspring (Table 1).

Table 1. *Plasma insulin secretion from isolated pancreas in vitro taken from control and LP offspring of rabbits*

(Mean values with their standard errors for eight control and five LP observations)

	Insulin secretion (ng/mg fresh pancreas per 30 min)					
Addition to medium	None	Glucose	None	Leucine	None	Arginine
Control offspring	4·56±0·55	4·73±0·81	2·11±0·54	2·86±0·79*	1·45±0·50	1·30±0·38
LP offspring	4·88±1·13	9·17±2·21*	4·25±0·87	6·79±1·64*	3·05±0·89	2·88±1·47

*Difference from appropriate control period: $P<0·05$.

LP offspring, offspring of rabbits given the low-protein diet.

The control offspring had a fasting plasma GH concentration of 6·3 ng/ml, which rose to a peak value of 12·3 ng/ml 30 min after the intraperitoneal administration of arginine (0·5 g/kg body-weight). The LP offspring had a raised fasting GH concentration of 27·4 ng/ml but failed to respond to the arginine challenge (Table 2).

Table 2. *Plasma growth hormone concentrations in control and LP offspring of rabbits before and after intraperitoneal injection of arginine* (0·5 *g/kg body-weight*)

(Mean values with their standard errors for six observations)

	Plasma growth hormone (ng/ml)			
Time after arginine injection (min)	0	30	60	90
Control offspring	6·3±2·1	12·3±2·2*	8·1±1·0	6·5±2·0
LP offspring	27·4±4·2††	29·9±5·8†	27·7±3·1†††	33·5±3·5††

*Difference from time 0: $P<0·05$.
Difference from control offspring: †$P<0·05$, ††$P<0·01$, †††$P<0·001$.
LP offspring, offspring of rabbits given the low-protein diet.

The results in LP offspring indicate impairment of pituitary function, such as occurs in post-natally induced protein-energy malnutrition (Turner, 1972), co-existing with enhanced insulin secretion such as is found in the offspring of diabetic mothers, and which may be a feature of early protein-energy malnutrition.

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The effect of casein and amino acids on glucose synthesis in sheep. By D. B. Lindsay and Caroline Dyke, *Department of Biochemistry, ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

In an earlier communication (Lindsay & Williams, 1971), it was reported that glucose production, as determined by isotope dilution, was significantly increased in sheep fed hourly, when additional protein (casein, 100 g/24 h) was infused into the abomasum. Judson & Leng (1973) who used a similar technique, but over a much shorter time, found a linear relation between the amount of casein hydrolysate infused into the abomasum and extra glucose produced. Their results suggest that much less glucose would be produced from this amount of casein than was observed by Lindsay & Williams.

We have therefore made further experiments of the same type and compared the effect of casein infusion with that of infusion of the equivalent amount of non-essential amino acids known to be present in casein for both short (5–10 h) and longer (40–60 h) periods of infusion. After 5–10 h infusion, extra glucose produced from casein was 9·4±3·8 (SEM) g/24 h (n=3) and from an amino acid mixture (alanine, glutamic acid, proline, aspartic acid, glycine, arginine and serine) 12·9±2·8 g/24 h (4) These results are similar to those obtained by Judson & Leng. After 40–60 h infusion of casein, glucose production was increased by 28±8·0 g/24 h, which is very similar to the mean value of 31 g/24 h obtained by us earlier. After infusion of amino acids, the corresponding value was 50±9 g/24 h. In most experiments there was some increase in rumen propionate during the infusion of casein or amino acids. About

20–30% of the extra glucose produced could have been derived from the estimated increase in the production rate of rumen propionate.

Though several alternatives could explain the greater effect of prolonged protein or amino acid infusion, our findings are consistent with the notion that some amino acids may be converted into other compounds before they are utilized by the liver for glucose synthesis. They suggest that in assessing the significance of amino acids for gluconeogenesis, at least in fed ruminants, short-term experiments may be inadequate.

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Preliminary studies on the suitability of field bean (*Vicia faba* L.) protein isolate for lambs and calves. By I. F. DUTHIE, D. G. EDWARDS and B. ROGERS*, *The Lord Rank Research Centre, High Wycombe, Bucks*, and R. J. ANDREWS and J. A. WRIGHT, *RHM Agriculture Ltd, Wimborne, Dorset*

It has been demonstrated in work with rats that a protein isolate prepared from the Throws M.S. field bean (*Vicia faba* L.) has a nutritional value which compares very favourably with soya-bean protein isolate (Duthie, Porter & Gadsby, 1972). Experiments with soya-bean protein isolate have indicated suitability for use in milk substitutes for calves (e.g. Porter & Hill, 1963; Gorrill & Nicholson, 1969).

A preliminary investigation to assess the acceptability and safety of field bean (FB) isolate for young ruminants was made with Suffolk × Clun Forest lambs. The lambs were removed from their mothers immediately after birth and were given 150 ml colostrum by bottle before the experimental diets were introduced. FB isolate was added to a commercial milk substitute at a rate of 100 g/kg and sodium caseinate was added in a similar way to act as a control. Each treatment was given to six lambs from 0 to 14 d of age.

In a second experiment 50 and 100 g FB isolate were introduced per kg in place of dried skim milk in diets for veal calves, the FB isolate being included on the basis of protein contribution. Each level of FB isolate was given to ten Friesian calves, with twenty control calves under semi-practical conditions of housing. The treatments were given from 0 to 14 weeks of age, after which the calves were slaughtered and graded.

The results of both experiments are summarized in the table. The FB isolate diet was well-accepted by the lambs and they showed no adverse reactions. Live-weight gain, food intake and food conversion ratio were not significantly different. Similarly, in the much longer calf experiment, there were no adverse reactions to the FB isolate nor any differences in acceptability, performance, killing-out percentage, colour, conformation or finish.

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The results were:

(Mean values with their standard errors where given)

Addition to diet	Wt gain (kg/d)	Food intake (kg milk powder/d)	Food conversion ratio (kg milk powder/ kg live-wt gain)
	Experiment 1 (lambs)		
Control	0·275±0·0408	0·275±0·0380	1·00±0·0152
100 g FB isolate/kg	0·322±0·0357	0·308±0·0229	0·98±0·0588
	Experiment 2 (calves)		
Control	1·00 ±0·069	1·80	1·80±0·065
50 g FB isolate/kg	1·02 ±0·022	1·79	1·76±0·125
100 g FB isolate/kg	0·91 ±0·056	1·81	2·04±0·120

At present there is considerable interest in alternative protein sources in milk substitutes for young animals, and these preliminary studies with lambs and calves indicate suitability for FB isolate in this type of application. It is suggested that results obtained from the lamb and calf, as neonatal animals, are of value in the nutritional evaluation and toxicological screening of this type of protein for human use.

We thank the Department of Agriculture, University of Reading, for valuable assistance with the lamb experiment.

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Contribution of microbial nitrogen to duodenal digesta in the ruminant.

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

Various methods have been used in attempts to measure the contribution of micro-organisms to the total protein entering the small intestine of the ruminant, but all the methods have deficiencies or limitations of one sort or another. We have compared values obtained by methods based respectively on the use of ribonucleic acid (RNA) and diaminopimelic acid (DAP) as markers.

A protozoa-free Friesian calf provided with a rumen and duodenal cannula was given several different diets all consisting of equal weights of hay and concentrates. Three sets of digesta collections were made after the calf had been given each diet for at least 2 weeks. RNA and DAP were determined in samples of mixed bacteria separated from rumen contents taken 4 h after feeding, and samples of whole duodenal digesta taken 1–2 h later. Results are presented in Table 1.

Table 1. *Composition of mixed bacteria and related duodenal samples (expressed as mg/g total non-ammonia nitrogen, NA-N) and calculated microbial N in the latter*

(Mean values with their standard errors)

	RNA-N		Diaminopimelic acid (DAP)-N		Microbial-N/ total NA-N in duodenal contents	
	Bacteria	Duodenal digesta	Bacteria	Duodenal digesta	RNA	DAP
Concentrates in diet	Source of digesta: Calf (protozoa-free)					
Flaked maize	121±7	72±11	5·8±0·8	3·7±0·7	0·60±0·08	0·63±0·07
Crushed oats	97±6	56±5	8·3±0·4	4·8±0·4	0·58±0·01	0·59±0·06
Rolled barley	101±8	80±7	9·5±0·5	6·9±0·6	0·79±0·08	0·73±0·03
Flaked maize+urea	92±1	54±5	8·5±0·2	4·3±0·7	0·59±0·06	0·50±0·11
	Source of digesta: Cow					
Dairy cubes	71±3	55±6	7·3±0·5	3·0±0·2	0·78±0·03	0·40±0·03

Values for the contribution of microbial nitrogen to total N were calculated assuming that the compositions of the bacterial samples were representative of the rumen micro-organisms and that the duodenal RNA and DAP were derived only from these micro-organisms. The good agreement found supported the validity of these assumptions for the calves.

Similarly related rumen bacterial and duodenal samples were obtained from a cow given diets of hay and concentrates except that total 72 h collections of duodenal digesta were made and representative samples of rumen bacteria were obtained during the collection period. Results for these experiments, also given in Table 1, showed a marked discrepancy between the two techniques for this animal.

Unless considerable amounts of dietary RNA survived rumen degradation in the cow experiments, and this seems unlikely in view of the rapid breakdown of nucleic acids found in the rumen (McAllan & Smith, 1973), it seems possible that the DAP method underestimated the microbial contribution in the cow because it took no account of the presence of protozoa.

We are grateful to Dr D. A. Corse for providing the samples of cow digesta.

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Digestibility of ^{15}N-labelled proteins in the small intestine of the ruminating calf. By D. N. SALTER and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Although microbial cells are usually an important source of protein for the ruminant, few direct determinations of the digestibility of microbial protein in the ruminant

have been made. Bird (1972) reported a value of 0·71 determined with one mixed sample of ^{35}S-labelled rumen bacteria.

A calf with simple duodenal and re-entrant ileal cannulas was used in a series of experiments during which it was given diets of tapioca and straw or flaked maize and hay. There were no marked differences in the results for the different diets, and determinations of the net digestibilities up to the ileum of total nitrogen compounds entering the duodenum (according to Smith & McAllan, 1971) were 0·663, 0·550, 0·630, 0·615, 0·666, 0·612 and 0·580 in a 4-month series of experiments. Determinations of true N digestibility were carried out in the same period using (a) samples of mixed rumen bacteria labelled with ^{15}N which had been harvested from the rumens of calves given [^{15}N]urea, and (b) ^{15}N-labelled wheat-leaf chloroplastidic protein (prepared by Mr N. W. Pirie, Rothamsted Experimental Station). These materials were incubated with pepsin (37°C for 3 h at pH 2), polyethylene glycol (PEG) was added, and the final digests, containing 0·3–0·9 g N, made up to 130–160 ml. A digest was infused into the duodenal cannula at a rate of 8–10 ml/min and samples of ileal digesta were collected in successive 50 g fractions for 6 h. ^{15}N was determined by an optical emission spectrometric method. The pattern of emergence of ^{15}N corresponded closely to that of PEG with the peak concentrations appearing in ileal samples at 3·5–4·5 h after infusion. From the mean ratios of ^{15}N: PEG in these samples the digestibilities of ^{15}N in the infusates were calculated. In a series of experiments, high and consistent values for the true digestibility of [^{15}N]—chloroplastidic protein were obtained (0·734, 0·760, 0·753, 0·816 and 0·782). A series of values for the digestibility of rumen bacterial ^{15}N, however, varied greatly 0·605, 0·696, 0·391, 0·382 and 0·439). This variation may have been related to the fact that for each infusion different batches of bacteria were used which had been obtained from the rumen of calves given diets containing varying proportions of urea and decorticated groundnut meal. The results support the view of Bergen, Purser & Cline (1967) that variation in microbial digestibility may have a marked effect on the utilization of N by the ruminant.

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The effect of dilution rate upon fermentation in the rumen. By D. G. Harrison, D. E. Beever and D. J. Thomson, *Grassland Research Institute, Hurley, Berks. SL6 5LR*

Hobson (1965) examined the effect of increased dilution rate upon the fermentation of rumen bacteria in vitro. Our experiments investigated the effects of altered dilution rates upon rumen fermentation in vivo.

Two mature wether sheep fitted with rumen cannulas were fed hourly on a ration of dried perennial ryegrass (1000 g/d) plus barley–fish-meal concentrate pellets (100

g/d). Water at rates of 0 (control), 4, 8 and 12 l/d was continuously infused into the rumens of both animals, and rumen dilution rates were determined by a modification of the method of Warner & Stacy (1968), using Cr—[2-^{14}C]EDTA. The infusion of water at 12 l/d increased the dilution rate by 10%; all other infusions gave dilution rates similar to that of the control. Thus the infusion of water had little effect on dilution rate.

The principal experiment examined the effects of infusing iso- and hypertonic solutions upon dilution rate. One of the above sheep, fed on the same diet, was infused intraruminally at the rate of 4 l/d with the following solutions in turn: (1) water, (2) artificial 'saliva' (McDougall, 1948) pH 6·9, osmotic pressure (OP) = 345 mosmol, (3) 'saliva' containing 40 g/l polyethylene glycol (PEG, mol. wt 1000) OP = 384, (4) 'saliva' containing 80 g/l PEG, OP = 422, (5) $NaHCO_3$ solution (25 g/l), pH 8·1, OP = 358, and (6) no infusion (control).

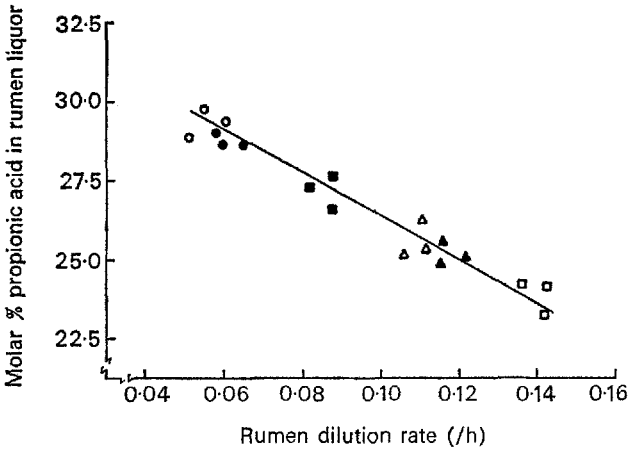

Fig. 1. Relationship between molar % of propionic acid in rumen liquor and rumen dilution rate in sheep infused intraruminally at 4 l/d with: ○, H_2O; ●, no infusion (control); △, artificial 'saliva'; ▲, 'saliva' containing 40 g/l PEG; □, 'saliva' containing 80 g/l PEG; ■, $NaHCO_3$ (25 g/l). Values were determined on three separate occasions.

The infusion of both 'saliva' (2) and $NaHCO_3$ (5) increased dilution rates (Fig. 1) and the addition of PEG to the 'saliva' (3, 4) increased the values still further. All rumen pH values were in the range 6·0–6·3, except with $NaHCO_3$ (pH 6·8). There was a highly significant ($P<0·001$) regression of the molar percentage of propionic acid in the rumen liquor (P) on the dilution rate (D): $P = 32·8–88·0\ D$; $r = -0·935$, $n = 17$.

The results indicate that dilution rate may be increased by intraruminal infusion of iso- or hypertonic solutions, and that the pattern of rumen fermentation for a given animal on a particular diet appears to be strongly influenced by dilution rate. These findings are in general agreement with the work of Hodgson & Thomas (1972).

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The effect of ruminal or postruminal digestion of lactose or fat on the voluntary intake and digestibility of dried grass by lambs. By PENELOPE C. BAILEY* and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB9 9SB*

When lambs fed to appetite on a barley-based concentrate were given a solution of lactose by bottle, so that the oesophageal groove was stimulated and the lactose entered the abomasum direct, the intake of concentrate decreased (Ørskov, Fraser & Pirie, 1973). The decrease in digestible energy consumed as dry concentrate was almost equal to the energy supplied as lactose, so total energy intake was unchanged. Though lambs fed on concentrates may respond to supplements in such a way as to keep energy intake constant, this may not be so for lambs fed on roughages.

In the present experiment the voluntary intake of dried grass by lambs was measured when the diet was supplemented with lactose or fat given either in the dry form or as a solution or emulsion. Two trials of latin square design (5×5) were made to study the effect of each type of supplement. In one square, lactose was given at one of two levels calculated to supply either 126 or 252 g/kg estimated dry-matter intake, in either the dry form from a trough or as a liquid from a bottle. In the other square a high-fat supplement (700 g beef tallow, 300 g skim-milk powder/kg) was given in quantities isoenergetic with the lactose supplements, to provide 70 or 140 g estimated dry matter intake.

Table 1. *Effect of lactose and fat supplements given in dry or liquid form on the voluntary intake of dry matter (DMI), digestibility of dry matter (DDM) from dried grass and on total intake of digestible energy (DE), including that of the supplements*

(Intakes expressed as: g or MJ/kg $W^{0.75}$ per d)

Supplementation		Lactose supplement			Fat supplement		
Method	Level	DMI (g)	DDM†	Total DE (MJ)	DMI (g)	DDM†	Total DE (MJ)
—	0	63·4	0·690	0·77	55·1	0·680	0·68
Dry	1	57·3	0·652	0·75	48·8	0·646	0·68
Dry	2	55·9	0·639	0·85	45·1	0·608	0·72
Liquid	1	59·8	0·679	0·83	54·3	0·671	0·75
Liquid	2	53·3	0·591	0·79	51·2	0·649	0·81
SEM		2·35	0·0166	0·032	2·67	0·0232	0·035

†Assuming complete digestion of the supplements.

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Some of the results are given in Table 1. Lactose given by either route reduced the intake of dried grass ($P<0.05$); with liquid feeding, this was particularly apparent at the high level of supplementation, when it was associated with severe scouring. Fat supplements given in the dry form also reduced grass intake ($P<0.01$), but when given as an emulsion direct into the abomasum they had no significant effect on grass intake and thus increased total digestible energy intake by about 15%.

The results confirm the adverse effects on intake and digestibility of roughages of giving soluble carbohydrates (Bowman & Huber, 1967), or fats (Bull, 1971), so that they enter the rumen, and suggest that if soluble carbohydrates or fats are given postruminally, some of the undesirable effects associated with their presence in the rumen can be avoided. This beneficial effect will, however, only be apparent if the supplements can be readily digested in the small intestine. The scouring observed with lactose given at a high level by bottle suggests that fat may be a better supplement in this respect.

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Utilization of dietary ammonium polyphosphate by growing wether lambs. By G. FISHWICK (introduced by R. G. HEMINGWAY), *Glasgow University Veterinary School, Bearsden, Glasgow*

Wether sheep (five/treatment; mean live weight, 39 kg) were fed on a basal diet consisting of about 0·14 kg chopped hay (1·5 g phosphorus, 11·0 g nitrogen/kg dry matter (DM)) and about 1·1 kg molassed sugar-beet pulp (SBP; 0·6 g P, 16·8 g N/kg DM) to which was added 4·6 g N/d as 10 g urea. These were given in two approximately equal portions/d. Comparisons were made between this diet and diets with the addition of 1·75 g P/d given as either (a) 10·94 g dicalcium phosphate (DCP, 160 g P/kg) or (b) as 6·25 g ammonium polyphosphate (APP; 280 g P and 125 g N/kg) when the urea addition was reduced to 8·3 g/d. After a 7 d introductory period with each diet, 7 d balances were conducted. The rather soft faeces were collected into polyethylene bags (Fishwick, 1973). A summary of the results is given in Table 1.

In the absence of added P the mean intakes of both hay and SBP were less because of a reduction in appetite which generally occurred 7–10 d after withdrawal of supplementary P. Addition of either form of supplementary P rapidly (2–3 d) restored appetite, and both P sources significantly increased the daily live-weight gain, the apparent retention of P and the blood P concentration, which was measured on the last day of each balance period.

Addition of either form of P also increased the apparent N retention. The experiment could not, however, be expected to differentiate between urea and APP as N

Table 1. *Mean dietary intake (fresh matter), live weight, live-weight gain, phosphorus and nitrogen balances, and concentrations of phosphorus, calcium and urea in the blood of the sheep*

	Treatment				
	A	B	C		
Supplement	None	DCP	APP	SEM	Significance
Intake (g):					
hay	0·11	0·13	0·16	—	—
SBP	1·04	1·10	1·16	—	—
Live wt (kg)	38·3	38·3	41·6	—	—
Live-wt gain (kg/d)	0·09	0·26	0·20	0·037	B,C>A*
Phosphorus (g/d):					
intake	0·72	2·53	2·57	—	—
retention	−0·22	+1·14	+1·15	0·088	B,C>A***
Nitrogen (g/d):					
intake	20·94	22·34	23·55	—	—
retention	+3·11	+5·63	+4·34	0·449	B>A***
Blood concentration of (mg/100 ml):					
P	2·67	6·21	6·33	0·354	B,C>A***
Ca	11·98	10·37	10·50	0·267	A,>B,C**
Urea	28·88	31·25	31·62	2·462	NS

SBP, sugar-beet pulp; DCP, dicalcium phosphate; APP, ammonium polyphosphate; NS, not significant. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

sources because when APP was given some 83% of the added N was still in the form of urea.

It is concluded that APP is a satisfactory form of dietary P for ruminants.

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Plasma lipoproteins of the suckling lamb compared with adult sheep.

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Adult and newborn sheep, in comparison with other ruminants, have low concentrations of plasma lipids, but in the neonatal lamb a relative hyperlipidaemia develops during the suckling period (Leat, 1967). To investigate in more detail the changes in plasma lipoproteins in the neonatal period, blood samples were taken at weekly intervals from seven Clun Forest lambs fasted for 18 h, and the plasma lipoproteins were isolated by flotation in the preparative ultracentrifuge (Hatch & Lees, 1968).

The concentration of plasma lipoproteins reached a maximum at 4 weeks *post partum* (Table 1) with values of up to 700 mg/100 ml being recorded; values then declined to near adult values at weaning (90 d *post partum*). High-density lipoprotein (density 1·063–1·210) was the largest component of plasma lipoproteins (52–69%) and chylomicrons (density<1·006) the smallest (<5%). Low-density lipoproteins (density 1·006–1·063) ranged from 9 to 23% during the neonatal period and were negatively correlated with very low-density lipoproteins.

Table 1. *Plasma lipoproteins (mg/100 ml) of sheep and neonatal lambs (mean of three determinations)*

Age (d)	Newborn	7	14	21	28	35	90	Adult
Chylomicrons	1·7	6·6	6·2	12·8	10·0	8·6	3·8	3·8
Very low-density lipoproteins	13·3	36·1	40·9	45·8	48·9	52·9	40·0	14·0
Low-density lipoproteins	21·7	76·5	105·0	112·1	143·2	112·7	62·8	70·5
High-density lipoproteins	71·4	288·7	386·3	352·4	394·7	391·3	185·0	286·5

The plasma lipoproteins of adult sheep consist mainly of high-density lipoproteins (70%) and low-density lipoproteins (17%), which can be separated at density 1·063. Chylomicrons (<1%) and very low-density lipoproteins (<10%) are minor components. On cellulose acetate electrophoresis two major lipoprotein bands are present, one corresponding to β_1-lipoproteins (low-density) and the other to α_1-lipoproteins (high-density).

In the plasma of suckling lambs a lipoprotein was present which was not detected in adult sheep. It is associated with the high-density lipoprotein fraction and has a density of 1·090 g/ml, compared with 1·138 g/ml for the high-density lipoprotein. This new lipoprotein is detected at 1 week *post partum* and persists to 7 weeks of age with a maximum at about 4 weeks *post partum.*

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Linolenic acid deprivation in mice. By J. P. W. RIVERS and B. C. DAVIDSON, *Nuffield Institute of Comparative Medicine, Zoological Society of London*

There is insufficient information about the importance of the essential fatty acids (EFA) in nutrition. Fatty acids of both the linoleate (or ω6) and linolenate (or ω3) series exhibit EFA activity and both are widely distributed in animal tissues (Crawford & Sinclair, 1972). The two series are not physiologically interconvertible and it is difficult, therefore, to accept the view that the EFA requirement can be

satisfied by linoleate alone. We wish to report some preliminary experiments on mice which may suggest a nutritional role for linolenic acid.

Matched groups of female albino mice were fed *ad lib.* from weaning on either a linolenate-poor diet containing safflower-seed oil (SSO) or a linolenate-supplemented diet containing soya-bean and linseed oils (SBOL). The diets were adequate in linoleic acid and differed only in the nature of the fat. No clear difference in growth between the groups was observed in 90 d of feeding. A difference in growth gradually became evident after this time, but no clinical abnormalities were observed.

However, young bred from the SSO group at 60 d weighed, at weaning, 17% less than offspring of control animals, and this difference persisted after weaning onto the parental diet.

Respiratory exchange during a 24 h fast was measured in young, growing female mice by a gravimetric method (Haldane, 1892). The results, shown in Table 1, indicate that SSO animals had a higher fasting metabolic rate than controls.

Table 1. *Fasting metabolism in mice previously given SSO and SBOL diets*

	No. of determinations	Metabolic rate (MJ/d) per animal	per kg body-wt	per $kg^{0.75}$
SSO diet	5	0·034	1·145	0·473
SBOL diet	8	0·031	0·974	0·412
Ratio, SSO value:SBOL value		1·10	1·18	1·15
P		<0·1	<0·01	<0·01

SSO, safflower-seed oil; SBOL, soya-bean and linseed oils.

Despite the obvious parallel with the increased metabolic rate of EFA deficiency (Alfin-Slater & Aftergood, 1968), these results do not in themselves justify the assumption that the SSO animals were exhibiting a deficiency state. They do, however, suggest that long-term deprivation of linolenic acid can produce metabolic changes which affect second-generation animals. We suspect there is some evidence for suggesting that more attention should be paid to the balance of EFA in semi-purified diets.

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Linolenic acid deprivation in Capuchin monkeys. By A. J. Sinclair, R. N. T.-W. Fiennes, A. W. M. Hay, G. Watson, M. A. Crawford and M. G. Hart, *Nuffield Institute of Comparative Medicine, Zoological Society of London*

During the course of an investigation into the vitamin D requirements of primates, eight male Capuchin monkeys (8–12 months old) were maintained on a purified diet (Herbert, 1971), supposedly supplying the full range of nutrients. The sole source of fat was maize oil, which supplied 12·7% of the total energy (linoleic acid 7·33% and linolenic acid 0·09% of the total energy). After 20–30 months on the

diets, the monkeys, whatever their vitamin D status, developed severe skin lesions similar to those observed in other species maintained on fat-free diets (Holman, 1968). Because maize oil is rich in linoleic acid and poor in linolenic acid, it occurred to us that these animals might be deficient in linolenic acid.

Two of the Capuchins had been killed during the experiment. Of the remainder, two developed behavioural abnormalities, gnawing insistently at their genitalia, which made their destruction necessary. From them specimens were taken for biochemical analysis and histological studies. Blood samples and liver biopsy material were obtained from the remaining monkeys.

After liver biopsy, the monkeys were placed on a diet in which half the maize oil was replaced with linseed oil; this mixture provided 4·61% of the total energy as linoleate and 3·59% as linolenate. One monkey was fed on this diet for 6 d only, returned to the maize-oil diet and killed 50 d later. A second was fed on the maize-oil–linseed-oil diet for 56 d and killed. The third Capuchin received the maize-oil–linseed-oil diet for 56 d and returned to the maize-oil diet for 142 d. The fourth Capuchin received the maize-oil–linseed-oil diet for 198 d.

Addition of linseed oil to the diet resulted in progressive resolution of the skin lesions; when the linseed oil was again withheld, the skin lesions reappeared. The animals given maize oil only showed, both at biopsy and autopsy, a uniformly distributed fatty infiltration of the liver, seen, histologically, as large fat droplets in the liver parenchyma, and, biochemically, as a total lipid content of from 300 to 600 g/kg dry matter. The histological changes disappeared when linseed oil was added to the diet and the liver lipids in the two treated animals were reduced to 190 and 240 g/kg dry matter. Even in the Capuchin which received linseed oil for only 6 d, fat was not found in excessive amounts 50 d later, although excessive amounts were noted at biopsy immediately before treatment with the linseed-oil diet. However, in this animal the skin lesions did not resolve.

Preliminary results for two further Capuchin monkeys and two patas monkeys have confirmed the above findings.

Although more detailed studies are clearly required, these findings suggest that linolenic acid or some other component of linseed oil is an essential nutrient for these primate species.

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Lipid composition of human milk: comparative studies on African and European mothers. By M. A. CRAWFORD and PAMELA STEVENS, *Department of Biochemistry, Nuffield Institute of Comparative Medicine, Zoological Society of London*; and P. MSUYA and A. MUNHAMBO, *Department of Biochemistry, Medical School, Dar-es-Salaam, Tanzania*

The main component of milk lipid is triglyceride. It is accepted that the fatty acid content of milk triglyceride can vary in relation to diet, but the potential

nutritional significance of qualitative and quantitative aspects of the fatty acid content is seldom considered. In many instances the only essential fatty acid reported in human milk is linoleic acid.

We have studied sixty-four milk samples from European and Tanzanian mothers during the first 6 months of lactation. Variations in fatty acid content occurred between individuals and with time after parturition, but the triglycerides in all instances were found to contain several essential fatty acids, including linoleic (C18:2 ω6), linolenic (C18:3 ω3), arachidonic (C20:4 ω6) and docosahexaenoic (C22:6 ω3) acids. Although the total amounts of the C_{20}–C_{22} polyenoic acids were small, they were however important when considered in the context of triglyceride as the main milk constituent and the high biological activity of the C_{20}–C_{22} polyenoic acids, which have special relevance to brain growth (Crawford & Sinclair, 1972; Sinclair & Crawford, 1972).

A study was made of the milk from mothers in East Africa where the children could be considered to be 'at risk' to marasmus and kwashiorkor. In some instances the C_{16}–C_{20} fatty acids were partly replaced by a substantial increase in acids of a 10–14 carbon chain-length. We also observed a variation in total milk fat, which in some individuals was below 20 g/l milk.

The substitution of the long-chain by short-chain fatty acids in milk has been observed in experiments where lipid undernutrition in rats led to increased mortality, reduced body size and decreased brain cell number in their pups (Sinclair & Crawford, 1973). These findings and the distortion of human milk fatty acid patterns in those at risk to undernutrition raises the question as to whether or not the alleged relationship between malnutrition and mental retardation (Cravioto, Pinero, Arroyo & Alcalde, 1969) may be just as much a function of lipid undernutrition as it is of so-called 'protein malnutrition'.

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Effect of glycerol on the incorporation of fructose and glucose into hepatic triglyceride of rats. By M. WUSTEMAN* and I. MACDONALD, *Department of Physiology, Guy's Hospital Medical School, London SE1 9RT*

Zakim, Pardini, Herman & Sauberlich (1967) have shown that high-fructose diets produce a greater concentration of triglyceride in the serum and liver of rats than high-glucose diets. Fructose has been shown to make a greater contribution to glyceride formation in rat liver than glucose (Nikkila, 1966). This greater contribution has been found to be predominantly to the glycerol moiety (Bar-on & Stein, 1968).

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Glycerol itself induces a significant rise in serum triglyceride concentrations in man (Macdonald, 1970), and in rats chronic glycerol feeding results in a marked increase in liver triglyceride (Nikkila & Ojala, 1964).

As α-glycerophosphate is a common precursor from fructose and glycerol in triglyceride synthesis, it has been proposed (Macdonald, 1970) that it is the concentration of this common intermediate that brings about the similar effect on triglyceride synthesis in the liver. It was therefore decided to examine the effect of feeding with glycerol on the incorporation of fructose into triglyceride and to compare this with the effect on glucose incorporation.

Twelve young (200 g) male rats of the Wistar strain were given 15% glycerol in water as drinking fluid in addition to their normal chow diet. After 1 week, and following an overnight fast, two rats were given, by intraperitoneal injection, a dose of either [U-C^{14}]fructose or [U-C^{14}]glucose. Two hours later the animals were killed and the livers removed. The remaining rats were killed, in pairs, at weekly intervals, the same procedures having been followed. After extraction from the liver, the lipids were separated by thin-layer chromatography, and the radioactivity of the triglyceride was determined. The triglyceride was saponified and the fatty acids were extracted for determination of radioactivity.

The results confirmed the earlier observations of Nikkila & Ojala (1964) and Nikkila (1966) that the chronic glycerol ingestion increased the amount of triglyceride found in the liver. At all times the specific activity from [C^{14}]fructose was greater than that from glucose. The specific activity of the triglyceride from radioactive fructose did not alter with time on the glycerol diet, but a significant decrease ($P<0{\cdot}005$) in the specific activity of the triglyceride from radioactive glucose was seen. There was a significant increase with time ($P<0{\cdot}05$) in the amount of radioactive fructose incorporated into triglyceride, expressed in counts/min per g liver, which was not seen for glucose.

Finally, in these animals, which were fasted, at no time did the proportion of radioactivity in the fatty acid moiety of the liver triglyceride exceed 9%.

These results would be consistent with the hypothesis that glycerol and fructose induce hepatic triglyceride synthesis via the same metabolic pathway.

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The effect of a high-carbohydrate diet on skin lipogenesis in the rat.

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It has been shown that the nature of the dietary carbohydrate affects the amount of triglyceride on the surface of the skin in man (Llewellyn, 1968) and that a high-

carbohydrate diet increases the amount of skin surface lipids in rats (Nikkari, 1965).

In vitro studies on rats in this laboratory using radioactive substrates have shown that this increase in lipid output is associated with an increased lipid synthesis by the skin. It was therefore decided to study the effect of diets containing glucose or fructose on skin lipogenesis from carbohydrate.

Male and female Wistar rats weighing between 180 and 200 g were put on a diet containing (g/kg): glucose or fructose 700, calcium caseinate 180, cellulose 30, salts 40, yeast 40 and sunflower-seed oil 10. At intervals up to 5 weeks rats were killed and a portion of skin from the back was removed, stripped of adipose tissue and hair and incubated with either [^{14}C]glucose or [^{14}C]fructose.

Skin from rats on a control diet was similarly treated.

The total lipid in the skin was extracted using chloroform–methanol 2:1 (v/v). A portion was removed for thin-layer chromatography and the radioactivity in the cholesterol, triglyceride, sterol and wax esters was assayed. The radioactivity in the total lipid was also measured.

On the glucose diet, compared with the control diet, there was an increased incorporation of [^{14}C]glucose into total lipids and this effect was noticed throughout the period of the diet in both sexes. A similar effect was seen using [^{14}C]fructose with rats on the fructose diet.

On the fructose diet, but not on the glucose diet, there was a significant increase in the incorporation of radioactivity in free cholesterol.

The incorporation of radioactivity in the sterol and wax esters increased in rats fed on the glucose diet but not in those on the fructose diet.

The radioactivity in the triglyceride fraction, on both the experimental diets, showed an initial rise followed by a fall after a few weeks.

From these studies it seems that dietary factors may have an influence on skin lipid synthesis in the rat.

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