ABSTRACTS OF COMMUNICATIONS

The Two Hundred and Seventy-ninth Scientific Meeting of the Nutrition Society was held at the Royal Society of Medicine, Wimpole Street, London W1M 8AE, on Friday, 16 May 1975, at 10.30 hours, when the following papers were read:

Responses of growing pigs to combinations of essential amino acids. By M. F. FULLER, R. M. LIVINGSTONE and I. MENNIE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Fifteen female pigs weighing about 25 kg were given for 10 d a diet containing 860 g dry matter and 18 g nitrogen/kg, consisting of barley with added vitamins and minerals. Each day for the next 10 d one combination of four of the following amino acids (g/kg) was added: L-lysine 4·0, DL-methionine 1·0, L-threonine 1·5, L-isoleucine 2·5 and L-tryptophan 0·6. For the final 10 d each mixture was completed by the addition of the missing amino acid. All these diets were given throughout at a daily rate of 120 g/kg body-weight$^{0.73}$. Urinary urea excretion was measured daily by total collections using urethral catheters. A new equilibrium rate of excretion was reached within 4 d of changing amino acid concentrations: the differences between successive periods in the mean rates after equilibration were:

Supplementary amino acid omitted in period 2 ...	Change in daily urea excretion (g)					SE of difference
	Lys	Met	Ileu	Thr	Try	
Effect of:						
1. Adding four amino acids (period 2–1)	+ 0·9	− 9·3	−11·5	− 4·1	−10·1	1·1
2. Adding 5th amino acid (period 3–2)	−11·1	− 1·8	+ 0·3	− 6·8	− 2·1	1·9
3. Adding five amino acids (period 3–1)	−10·2	−11·5	−11·2	−10·9	−12·1	2·2

Lys, lysine; Met, methionine; Ileu, isoleucine; Thr, threonine; Try, tryptophan.

Addition of amino acid mixtures devoid of methionine, isoleucine or tryptophan produced reductions of about 10 g/d in urea output, none of which was increased by subsequent addition of the missing amino acid. The mixture lacking lysine produced no response: the full change in urea output was obtained when lysine was subsequently added. The mixture containing no threonine gave an intermediate response, confirming earlier demonstrations of its role as second-limiting in barley protein (Fuller, Livingstone & Mennie, 1974; Taylor, Cole & Lewis, 1974). The final rate of urea excretion was on average 3 g/d (range 1–6): N retention was 16–20 g/d. The absence of responses to methionine, tryptophan and isoleucine throws doubt on currently used estimates of requirements for these amino acids (Agricultural Research Council, 1967).

https://doi.org/10.1079/PNS19750054 Published online by Cambridge University Press

The generosity of Ajinomoto Inc. (Japan) in donating the amino acids is gratefully acknowledged.

REFERENCES

Agricultural Research Council (1967). *The Nutrient Requirements of Farm Livestock No. 3. Pigs* London: Agricultural Research Council.
Fuller, M. F., Livingstone, R. M. & Mennie, I. (1974). *Proc. Nutr. Soc.* **33**, 91A.
Taylor, A. J., Cole, D. J. A. & Lewis, D. (1974). *Proc. Br. Soc. Anim. Prod.* **3**, 111.

An improved method for the determination of the biological availability of lysine in proteins using the micro-organism *Tetrahymena pyriformis*.

By N. D. SHEPHERD, T. G. TAYLOR and D. C. WILTON, *Department of Physiology & Biochemistry, University of Southampton, Southampton SO9 3TU*

Of the methods that have been developed for estimating the nutritive value of proteins, the microbiological assay using *Tetrahymena pyriformis* appears to have considerable potential for development. This micro-organism is capable of hydrolysing intact proteins and has broadly the same requirements for amino acids as higher animals, including lysine and methionine, which are often limiting in diets. In practice, however, it has proved difficult to estimate growth accurately. Furthermore, the assay underestimates amino acid availability in some proteins even after pretreatment with proteolytic enzymes.

In an attempt to overcome these problems a new method of estimating growth has been developed based on the determination by gas–liquid chromatography of tetrahymanol, the characteristic pentacyclic terpene synthesized by this protozoan (Mallory, Gordon & Conner, 1963; Thompson, Bambery & Nozawa, 1971).

Preliminary work had shown that there was a linear relationship between cell number and the tetrahymanol content of the extracted cells. The availability of lysine in a variety of different proteins was then determined using this method. The protein sources were predigested with pronase (a proteinase obtained from Streptomyces species) and extracted with diethyl ether to remove sterols. The assay conditions were similar to those used by Shorrock & Ford (1973) but the concentration of amino acids in the medium was reduced and the trace element solution was replaced by that of Holz, Erwin, Rosenbaum & Aaronson (1962).

The assays were carried out in capped test-tubes resting in a rotating drum in an incubator at 26° for 3–4 d and the cells were harvested by centrifugation. Tetrahymanol was extracted by the method of Thompson, Bambery & Nozawa (1972), using an internal standard of β-amyrin, and dissolved in diethyl ether for chromatography. The concentration of available lysine in the protein was calculated by slope ratio analysis using free lysine as a reference. Total lysine after acid hydrolysis was measured in an amino acid analyser (Model 5AH; Jeol, London). The results obtained were:

(Mean values with their standard errors; no. of determinations in parentheses)

	Total lysine (mg/g)		Available lysine (mg/g)		Availability ratio
	Mean	SE	Mean	SE	
Casein	75·1	1·2(7)	70·9	0·4(3)	0·94
Soya-bean protein	50·1	1·0(7)	43·9	2·4(5)	0·88
Maize gluten protein	11·3	0·4(8)	10·0	0·9(5)	0·88
Freeze-dried lucerne juice coagulum	31·7	0·8(8)	27·6	1·3(5)	0·87
Meat-and-bone meal	18·6	0·6(7)	11·1	0·6(5)	0·60
Fish meal	46·1	0·3(7)	40·3	1·4(5)	0·87
Heat-damaged	44·6	0·8(7)	32·2	1·2(9)	0·72
Severely heat-damaged	39·1	0·7(4)	19·3	0·7(4)	0·49

The results of chick bio-assays for available lysine in a number of different proteins have given results consistent with those obtained by the microbiological technique.

We wish to thank the Agricultural Research Council for a grant in support of this work.

REFERENCES

Holz, G. G. Jr, Erwin, J. A., Rosenbaum, N. & Aaronson, S. (1962). *Arch Biochem. Biophys.* **98**, 312.
Mallory, F. B., Gordon, J. T., & Conner, R. L. (1963). *J. Am. chem. Soc.* **85**, 1362.
Shorrock, C. & Ford, J. E. (1973). In *Proteins in Human Nutrition*, p. 207 [J. W. G. Porter and B. A. Rolls, editors]. London and New York: Academic Press.
Thompson, G. A. Jr, Bambery, R. J. & Nozawa, Y. (1971). *Biochemistry, Easton* **10**, 4441.
Thompson, G. A. Jr, Bambery, R. J. & Nozawa, Y. (1962). *Biochim. biophys. Acta* **10**, 630.

The energy value to sheep of a mixed grass silage. By J. S. SMITH, F. W. WAINMAN and P. J. S. DEWEY, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The conservation of forage as silage is being actively encouraged because the losses of nutrients involved are usually less than those in haymaking, it is less weather-dependent and the energy costs are less than those for artificial drying. However animals fed on silage often produce less gain or milk than expected or indeed predicted.

There have been few measurements of the net energy value of silages using animal calorimetry and there are none reported in the literature for UK silages. The experiment reported was the first of a series of determinations aimed to improve the prediction of animal performance from laboratory analysis of silage.

The silage used was prepared in 1974 without additives from the first harvest from a mixed grass–clover sward; it was collected from the farm in sacks, mixed, weighed into daily rations and stored at −20° until required. Two daily amounts of silage were weighed, estimated maintenance and twice this amount (dry matter (DM) basis). Two sheep were offered the high ration followed by the low, and two others were offered the low ration first; each feeding period was of 4 weeks duration. Closed-circuit respiration chambers (Wainman & Blaxter, 1969) were used to make measurements of fasting heat production before and after the feeding periods, and to measure the gaseous exchange over the final 4 d of each feeding

period. Excreta collections were made for the last 6 d of each feeding period. Brouwer's (1965) formula was used to calculate heat production from the gaseous exchange and urinary nitrogen excretion.

The mean daily N and energy balances of the four sheep were:

	Low level of feeding		High level of feeding	
DM intake (g)	615		1228	
N metabolism (g)				
Intake	17·95		35·84	
Faeces	4·43		9·54	
Urine	13·40		19·68	
Retention	0·12		6·62	
Energy metabolism (MJ)		(%)		(%)
Intake	12·12	100	24·19	100
Faeces	3·27	27·0	7·20	29·8
Urine	0·84	6·9	1·35	5·6
Methane	1·04	8·6	1·87	7·7
Metabolized	6·97	57·5	13·77	56·9
Heat	7·74		11·32	
Retention	−0·77		+2·45	
SE of retention	0·26		0·41	

The ratios of metabolizable energy (ME):digested energy, 0·79 at the low level of feeding and 0·81 at the high, were similar to those given by the Agricultural Research Council (1965). ME, at 11·27 MJ/kg DM, was unchanged by increasing intake, and this value is near the mean for sixteen other silages from the same area and season. The net availability of ME for fattening was 42·4% and the net energy for fattening 4·78 MJ/kg DM. These values are similar to those for good-quality first-harvest dried grass (crude protein (N×6·25) 111, crude fibre 261 g/kg) of 11·79 and 5·19 MJ/kg DM respectively. Energy losses as methane were also similar (Wainman, Blaxter, Smith & Dewey, 1970).

The results obtained in this experiment accord well with those of Ekern & Sundstøl (1974) for a Norwegian grass silage.

REFERENCES

Agricultural Research Council (1965). *The Nutrient Requirements of Farm Livestock* No. 2, *Ruminants*. London: Agricultural Research Council.
Brouwer, E. (1965). *Publs Eur. Ass. Anim. Prod.* no. 11, p. 441.
Ekern, A. & Sundstøl, F. (1974). *Publs Eur. Ass. Anim. Prod.* no. 14, p. 221.
Wainman, F. W. & Blaxter, K. L. (1969). *Publs Eur. Ass. Anim. Prod.* no. 12, p. 429.
Wainman, F. W., Blaxter, K. L., Smith, J. S. & Dewey, P. J. S. (1970). *Publs Eur. Ass. Anim. Prod.* no. 13, p. 17.

Field-bean (*Vicia faba* L.) protein in feeds for preruminant calves. By J. W. SISSONS and R. H. SMITH, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Earlier work has indicated that after preruminant calves have received a number of test feeds containing certain soya-bean products as the sole protein source they

may develop abnormalities in digesta movement and nutrient uptake (Smith & Sissons, 1975). The possibility of similar disorders developing in calves given feeds prepared from isolated field-bean (*Vicia faba* L.) protein (FB isolate; Rank Hovis McDougall Ltd, High Wycombe) was examined in animals with abomasal cannulas and re-entrant intestinal cannulas in either the proximal duodenum (A, two calves) or distal ileum (B, two calves). Calves were reared on whole milk but received test synthetic feeds at intervals of 2–3 d from about 8 weeks of age. Test feeds were prepared, and given by abomasal infusion, as described by Smith & Sissons (1975) except that some contained FB isolate (41 g/kg) as the sole protein source. FB isolate-feeds were given on five to eight occasions and compared with casein-feeds which were also given periodically.

Flow of digesta components from the abomasum, measured in the A calves as described by Smith & Sissons (1975), did not change with successive FB isolate-feeds. Mean recoveries at the duodenum up to 2·5 h after an FB isolate-feed expressed as proportions (g/g) of intake (±SEM) were 0·59±0·06, 0·47±0·05 and 0·36±0·06 for total digesta, marker polyethylene glycol (PEG) and nitrogen respectively: corresponding values up to 6 h after the feed were 1·00±0·04, 0·70±0·02 and 0·70±0·05 respectively. Recoveries after a casein-feed were similar to those reported earlier (Smith & Sissons, 1975) and differed markedly from those after an FB isolate-feed only in showing a greater hold-up of N relative to PEG in the abomasum.

Mean values (±SEM), measured in the B calves, for transit time of a marker through the small intestine (h), average rates of total digesta flow for periods of 3 h and 21 h following the arrival of food residues at the ileal cannula (g/h) and net absorption of dietary N up to the ileal cannula (g/g) were 3·7±0·7, 67±14, 42±11 and 0·82±0·04 respectively after an FB isolate-feed (no change with successive feeds) and 3·9±0·2, 104±10, 42±7 and 0·86±0·01 respectively after a casein-feed.

Digesta flow and N digestibility did not differ markedly between feeds prepared from FB isolate and casein and there was no indication that FB isolate-feeds caused a development of digestive disorders analogous to those caused by feeds prepared from certain soya-bean products.

REFERENCE

Smith, R. H. & Sissons, J. W. (1975). *Br. J. Nutr.* **33**, 329.

Food intake control in zinc-deficient rats of the Zucker-Zucker strain.

By J. K. CHESTERS, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Eight weanling obese rats of the Zucker-Zucker strain and eight control, non-obese rats of the same strain were offered the diet described by Williams & Mills (1970) supplemented with zinc for 5 d and then without Zn for a further 27 d. An abrupt reduction in growth rate and food intake was observed in both groups after 5 d on the Zn-deficient diet (day 10 of experiment) (Table 1).

Table 1. *Weight range, mean food intake and growth rate of Zucker-Zucker rats offered diets adequate and inadequate in zinc*

(Mean values with their standard errors)

Period of analysis	Rats	Initial body-weight (g) Mean	SE	Final body-weight (g) Mean	SE	Food intake (g/d) Mean	SE	Growth rate (g/d) Mean	SE
Days 1–9 (normal growth)	Obese	83	4	145	5	15·8	0·3	8·0	0·2
	Control	68	1	106	1	9·8	0·2	5·0	0·2
Days 10–20 (development of Zn deficiency)	Obese	145	6	166	4	Transitional from values for growing rats to new stable values for Zn-deficient animals			
	Control	106	3	115	3				
Days 21–32 (established Zn deficiency)	Obese	165	7	168	4	7·4	0·2	0·1	0·1
	Control	115	3	118	3	7·4	0·3	0·3	0·2

During the period when growth was not restricted by Zn supply, the fatty rats showed the expected increase in food intake and body-weight gain compared with the controls but when growth was prevented by lack of Zn the mean daily intakes of two groups were identical despite the large differences in their body-weights.

Table 2. *Body composition of obese and control rats after 27 d on the zinc-deficient diet*

(Mean values with their standard errors)

Rats	Body-weight (g) Mean	SE	Dry matter (g) Mean	SE	Fat (g) Mean	SE	Non-fat dry matter (g) Mean	SE
Obese	168	6	97·3	1·0	65·5	1·0	31·8	0·8
Control	118	3	40·2	0·6	7·7	0·4	32·5	0·5

The simultaneous reduction in the rate of body-weight gain of the two groups on day 10 and the similarity of their final fat-free dry weights (Table 2) suggests that they responded similarly to Zn deficiency. However, during the transition from normal growth to the period when mean daily body-weight increase was not statistically significant, the mean body-weight gain of the obese group was 12 g greater than that of the controls. The lean body-weights of obese and lean rats are similar over the age range of the present experiment when both groups are given a nutritionally adequate diet *ad lib.* (Radcliffe, Webster, Dewey & Atkinson, 1975). One may therefore assume that both groups had the same lean body-weight on day 9 and since it was also the same between groups on day 32 the difference in weight gain of the two groups during the transition period was probably associated with an increase in lipid reserves of the obese strain.

Between days 21 and 32 both groups maintained virtually a constant weight with identical food intakes suggesting that lean body-weight rather than total body-weight was the prime determinant of maintenance requirement.

REFERENCES

Radcliffe, J. D., Webster, A. J. F., Dewey, P. J. S. & Atkinson, T. (1975). *Proc. Nutr. Soc.* **34**, 53A.
Williams, R. B. & Mills, C. F. (1970). *Br. J. Nutr.* **24**, 989.

Maintenance requirement and adipocyte count of rats from large and small litters, at the same weight. By D. S. MILLER and A. WISE, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

The mean voluntary maintenance energy consumption is 590 kJ/kg body-weight $(W)^{0.75}$ per d (Brody, 1945), but a part of this intake may not be necessary; Miller & Payne (1963) estimated the minimum maintenance requirement as 450 kJ/kg $W^{0.75}$ per d. In order to investigate the mechanism whereby energy expenditure is controlled, it is useful to find simple models of animals that are maintaining weight on different energy intakes. This experiment was performed to show whether rats from large and small litters (Widdowson & McCance, 1960) could be used to provide such a model. Rats, suckled in litters of four or sixteen, were given stock diet until they were 6 months old, at which time the small-litter rats were 18% heavier than the large-litter rats. Groups of six rats (three male and three female) from each were caged separately and given weighed quantities of moistened, powdered stock diet. The diet of the small-litter rats was restricted for 7 d, at which time their average body-weight approximated that of the large-litter rats. Then food was given so that each rat maintained weight on a minimum amount of food. After a week, the energy intake remained stable for a further 5 d at 446 ± 9 kJ/kg $W^{0.75}$ per d and 499 ± 12 kJ/kg $W^{0.75}$ per d ($P<0.005$) for the reduced-weight, small-litter and large-litter rats respectively. Gross differences in carcass composition were not apparent; for example, both groups had the same lean body-weight and similar fat contents. These phenomena are similar to those observed in man, where some long-term slimmers also show a reduced maintenance requirement (Miller & Parsonage, 1975).

Adipocytes were counted using a Coulter counter after osmium fixation. Assuming samples of perigenital fat to be representative of all adipose tissue, small-litter rats contained more adipocytes per rat, both before (1.69×10^8) and after weight reduction (1.60×10^8) compared with large-litter rats (1.36×10^8) ($P<0.001$). This agrees with the findings of Knittle & Hirsch (1968), and furthermore agrees with the work of Hollenberg & Vost (1968), who used radioactive thymidine to show that mature adipocytes are not destroyed when weight is reduced.

REFERENCES

Brody, S. (1945). *Bioenergetics and Growth.* New York: Reinhold Publishing Corp.
Hollenberg, C. H. & Vost, A. (1968). *J. clin. Invest.* **47**, 2485.
Knittle, J. L. & Hirsch, J. (1968). *J. clin. Invest.* **47**, 2091.
Miller, D. S. & Parsonage, S. (1975). *Lancet* **i**, 773.
Miller, D. S. & Payne, P. R. (1963). *J. theor. Biol.* **5**, 398.
Widdowson, E. M. & McCance, R. A. (1960). *Proc. R. Soc.* B **152**, 188.

Plasma lipids and lipoproteins of some herbivorous mammals. By W. M. F. LEAT and CHRISTINE A. NORTHROP, *ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*, and D. M. JONES, *Zoological Society of London, Whipsnade Park, Dunstable, Beds. LU6 2LF*

The mode of digestion of dietary components by a herbivorous animal is dependent on the anatomy of its digestive tract. In simple-stomached herbivores

(e.g. horse, rabbit), digestion of lipids presumably takes place in the small intestine as in other simple-stomached mammals. In herbivores with complex stomachs (e.g. cow, sheep) hydrolysis of dietary lipids takes place in the rumen. This is associated with hydrogenation and complex changes in the chemical composition of the absorbed fatty acids (see Dawson & Kemp, 1970). It is not known whether these differences in digestive physiology have any effect on the subsequent mode of transport of lipids in the plasma. To investigate this point the plasma of a number of herbivorous mammals from different families was analysed by electrophoresis and ultracentrifugation to determine the relative proportions of low-density (LDL) and high-density lipoproteins (HDL) (Table 1).

Table 1. *Plasma lipids and lipoproteins of some herbivorous mammals, and of man for comparison*

Species		Low-density lipoproteins (%)	High-density lipoproteins (%)	Plasma lipids (mg/l) Cholesteryl esters	Plasma lipids (mg/l) Phospholipids	Stearic acid in plasma triglycerides (mg/g)
Perissodactyla						
Wild horse	(*Equus przewalski*)	28·1	71·9	418	1154	36
Mountain zebra	(*Equus zebra*)	11·4	88·6	1199	1262	41
White rhinoceros	(*Diceros simus*)	100	0	1238	585	62
Indian rhinoceros	(*Rhinoceros unicornis*)	100	0	748	608	46
Artiodactyla						
European bison	(*Bison bonasus*)	22·8	77·2	656	666	143
Domestic ox	(*Bos taurus*)	10·3	89·7	1675	1296	459
Musk ox	(*Ovibos moschatus*)	28·8	71·2	750	755	338
Domestic sheep	(*Ovis aries*)	21·9	78·1	542	491	328
Domestic goat	(*Capra hircus*)	21·3	78·7	1367	1133	371
Wapiti	(*Cervus elaphus*)	17·0	83·0	1199	535	455
Moose	(*Alces alces*)	12·6	87·4	873	602	121
Bactrian camel	(*Camelus bactrianus*)	100	0	614	361	135
Primata						
Man	(*Homo sapiens*)	71·9	28·1	2946	2267	57

In most of the herbivores studied so far, HDL was the predominant lipoprotein, and there was no apparent relationship between the type of digestive physiology and the distribution of plasma lipoproteins. In the Perissodactyla, HDL predominated in the family Equidae but was absent in the family Rhinocerotidae, where a complex lipoprotein was present having β-mobility on electrophoresis. In the Artiodactyla, HDL was the predominant lipoprotein in the families Cervidae and Bovidae, but in the Camelidae only LDL was detected (cf. Mills & Taylaur, 1971).

The plasma concentrations of cholesteryl esters and phospholipids were much lower than those found in man. The major fatty acid of the cholesteryl esters was linoleic acid, with particularly high values (73–78%) being found in the Perissodactyla. The stearic acid content of plasma triglycerides was high in many of the Artiodactyla, presumably because of hydrogenation in the rumen.

https://doi.org/10.1079/PNS19750054 Published online by Cambridge University Press

REFERENCES

Dawson, R. M. C. & Kemp, P. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.
Mills, G. L. & Taylaur, C. E. (1971). *Comp. Biochem. Physiol.* **40B**, 489.

Influence of dietary fat intake of the mother on composition of body fat of newborn guinea-pigs. By DIANA E. PAVEY and ELSIE M. WIDDOWSON, *Department of Medicine, Addenbrooke's Hospital, Hills Road, Cambridge*

The evidence in favour of direct transfer of fatty acids across the placenta is somewhat contradictory (Robertson & Sprecher, 1968). Studies have indicated that even in the guinea-pig, in which fatty acids are known to be transferred across the placenta and to make a significant contribution to the fat of the foetus, most fat is synthesized in situ (Chaikoff & Robinson, 1933). The present investigation was designed to study the net effect of the fatty acid composition of the diet of the pregnant guinea-pig on the composition of the body fat of the foetus.

Fifteen female guinea-pigs were fed from the middle of pregnancy one of three diets: control diet with no added fat, control diet with maize oil, control diet with beef tallow. The fat was added to the powdered stock diet (Oxoid Modified Diet 18) at 200 g/kg, and casein was included to maintain the percentage of total energy coming from protein approximately equal to that of the control diet alone. The animals were in positive energy balance during the remainder of pregnancy on all diets, and the addition of fat to the diet did not affect the number of live young born, or their birth weights.

The young were killed at birth and the bodies analysed for total fat and fatty acid composition of the fat. The newborn of mothers given a diet containing fat had more fat in their bodies (mg/g body-weight) than the newborn of mothers fed the control diet: maize-oil diet 136·4 ($P<0·01$), beef-tallow diet 130·8 ($P<0·01$), control diet 93·6. There were significant differences in fatty acid composition of the fat which reflected the different distribution of fatty acids in maize oil and beef tallow:

	Fatty acid (mg/g body fat)					
Dietary fat	16:0	16:1	18:0	18:1	18:2	18:3
Maize oil	211	19	63	246	412	32
Beef tallow	263*	36*	90*	391*	155*	36
Control	284*	31*	101*	251†	219*†	86*†

*Differs significantly from value for maize-oil diet, $P<0·01$.
†Differs significantly from value for beef-tallow diet, $P<0·01$.

There were also significant differences in the amounts of unsaturated fatty acids in the average newborn guinea-pig. The total amount of the saturated fatty acids in the bodies of the young of mothers fed maize oil and beef tallow was not significantly different.

It is clear that fats or fatty acids are transferred across the placenta. The results

suggest that unsaturated fatty acids cross the placenta more readily than the saturated ones.

REFERENCES

Chaikoff, I. L. & Robinson, A. (1933). *J. biol. Chem.* **100**, 13.
Robertson, A. F. & Sprecher, H. (1968). *Acta paediat., Stockh.* Suppl. 183, 3.

Intake, digestion and production by dairy cows given protected lipids in early lactation. By J. A. BINES, J. E. STORRY and P. E. BRUMBY, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Protected lipids are being investigated as a possible means of increasing energy intake of cows at times of maximum demand such as early lactation. In a preliminary trial (Bines & Storry, 1974) it was shown that intake of lipids could be increased substantially if they were protected by encapsulation in formaldehyde-treated protein, thus preventing release and metabolism of fatty acids in the rumen. Energy demand in that trial was low, as the cows were giving little or no milk and no increase in energy intake was observed. Further investigations are now in progress with cows in early lactation to find out whether intake can be increased during this period, whether supplements are digested in and absorbed from the intestines and whether their presence interferes with the digestibility of other constituents of the ration.

Four levels, 0, 1·7, 3·3 or 5·0 kg/d, of a formaldehyde-treated mixture of crushed soya beans and tallow containing 400 g lipid/kg were given to four groups of four cows in a continuous feeding trial during the first 13 weeks of lactation. In addition the cows had *ad lib.* access to hay and conventional concentrates, the ratio, hay:total concentrate being maintained at about 25:75. Food intake and milk yield were recorded daily and the cows were weighed twice weekly. The digestibility of the ration was determined in a 10 d balance trial conducted near the end of the experimental period. Results are so far available for two cows on each of the treatments.

Total food intake rose steadily till the 7th week of lactation, after which it rose a little further in unsupplemented cows, remained steady in cows given 1·7 or 3·3 kg supplement, but fell markedly in cows given 5 kg supplement. Mean values for food intake and milk production over the 13-week trial were:

	Level of supplement (kg/d)			
	0	1·7	3·3	5·0
Mean daily food intake (kg)	17·1	17·2	14·4•	14·7
Maximum daily food intake (kg)	20·8	19·1	17·5	16·7
Total milk output (kg)	1968	2067	2129•	1752
Milk fat (g/kg) †	35·0	36·7	39·9	40·5
Milk protein (g/kg) †	29·2	28·9	28·2	30·1
Milk lactose (g/kg) †	46·8	45·0	44·3	45·0

•Probably underestimates: cows ill during weeks 4 and 5.
†Mean value for weeks 7–13.

https://doi.org/10.1079/PNS19750054 Published online by Cambridge University Press

Thus, the lower levels of supplementation raised milk yield by 5 and 8% respectively, and milk-fat yield by 17 and 23%; the high level of supplementation depressed both food intake and milk output.

The digestibilities of some constituents of the ration and the serum lipid levels found in the cows were:

	Level of supplement (kg/d)			
	0	1·7	3·3	5·0
Dry matter (DM) intake during balance (kg/d)	17·7	15·9	15·2	10·4
Hay content of DM (g/kg)	240	230	220	290
Digestibility				
DM	0·72	0·69	0·72	0·68
Energy	0·72	0·70	0·74	0·70
Nitrogen	0·64	0·63	0·63	0·67
Diethyl ether extract	0·60	0·79	0·84	0·85
Blood serum lipid (mg/l)				
Total lipid	4570	8160	9230	9400
Low-density lipoprotein lipid	490	820	1240	1080

REFERENCE

Bines, J. A. & Storry, J. E. (1974). *Proc. Nutr. Soc.* 33, 61A.

Effect of dietary sunflower-seed oil on the fatty acid composition and desaturase activity of tissues from congenitally obese rats. By K. W. J. WAHLE and J. D. RADCLIFFE, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Enhanced hepatic lipogenesis (Martin, 1974) results in excessive accretion of lipids in liver and adipose tissue of congenitally obese (Zucker) rats (see Bray & York, 1971). Compared with their lean litter-mates, obese rats have greater Δ9-desaturase activity in liver microsomes and their tissue lipids contain more palmitoleic acid (16:1) and oleic acid (18:1) and less linoleic acid (18:2) and arachidonic acid (20:4) (Wahle, 1974 and unpublished observations). The addition of increasing amounts of linoleic acid to normal rat tissue in vitro is associated with decreasing Δ9-desaturase activity (Uchiyama, Nakagawa & Okui, 1967), but increasing dietary linoleic acid causes decreased fatty acid synthesis (Du & Kruger, 1972).

In the present study two groups of Zucker rats, each of five obese and five lean litter-mates, aged 32 d, were fed to appetite for 32 d on a sucrose-based diet which contained 50 or 200 g sunflower-seed oil/kg (diets LS and HS respectively); the oil contained 650 g linoleic acid/kg. Liver microsomes were prepared for desaturase assays and the fatty acids of liver lipids and subcutaneous triacylglycerols were analysed by gas–liquid chromatography.

A decrease of 40–60% in Δ9-desaturase activity toward [1–^{14}C]stearate was found in liver microsomes from both obese and lean rats fed on the HS as compared with the LS diet. Addition of fatty acid 18:2 to liver microsomes from obese and lean rats fed on a conventional diet also decreased Δ9-desaturase activity. The ratios 16:1/16:0 and 18:1/18:0 in liver lipids and 16:1/16:0 in subcutaneous triacylglycerols were lower in obese and lean rats given the HS diet

https://doi.org/10.1079/PNS19750054 Published online by Cambridge University Press

Table 1. *Effect of dietary sunflower-seed oil on the fatty acid composition of liver lipids and subcutaneous triacylglycerols (mol/100 mol) of obese and lean Zucker rats*

(Mean values for five rats/group)

	Diet	Fatty acid					
		16:0	16:1	18:0	18:1	18:2	20:4
		Liver lipids					
Obese	LS	35·3	9·1	8·6	35·9	4·9	3·7
	HS	24·3	4·1	12·6	22·2	19·4	9·4
Lean	LS	20·8	3·5	16·8	17·2	14·2	17·4
	HS	15·6	0·8	16·8	11·4	26·5	17·2
		Subcutaneous triacylglycerols					
Obese	LS	30·0	9·5	4·8	37·4	13·4	0·3
	HS	22·9	5·2	3·5	29·8	33·5	0·6
Lean	LS	26·0	8·1	3·8	32·0	24·4	0·4
	HS	14·8	2·0	3·1	25·3	49·6	0·7

LS, 50 g sunflower-seed oil/kg diet; HS, 200 g sunflower-seed oil/kg.

than in those given the LS diet (Table 1). This again indicates a diminished Δ9-desaturase activity in rats given the HS diet. Increased proportions of 18:2 but not 20:4 were found in liver lipids of lean rats given the HS compared with the LS diet; by contrast, proportions of both 18:2 and 20:4 were increased in liver lipids of obese rats given the HS diet, though the proportion of 20:4 was 50% less than that in lean rats given either diet. The lipid content of liver from obese rats given the LS and HS diets was 126 and 81 mg/g wet weight respectively; the corresponding values for lean rats were 39 and 56 mg/g. The net conversion of 18:2 to 20:4 was, therefore, lower in the livers of obese rats as compared with their lean litter-mates. Diminished fat accretion in the liver of obese rats given the HS diet may be related to inhibition of fatty acid synthesis in situ: a lower incorporation of [1–^{14}C]acetate into lipids was found in liver homogenates from obese and lean rats given the HS as compared with the LS diet. Dietary 18:2 thus depresses fatty acid synthesis and Δ9-desaturase activity not only in lean rats, but also in congenitally obese rats.

J. D. R. was supported by a grant from the British Nutrition Foundation.

REFERENCES

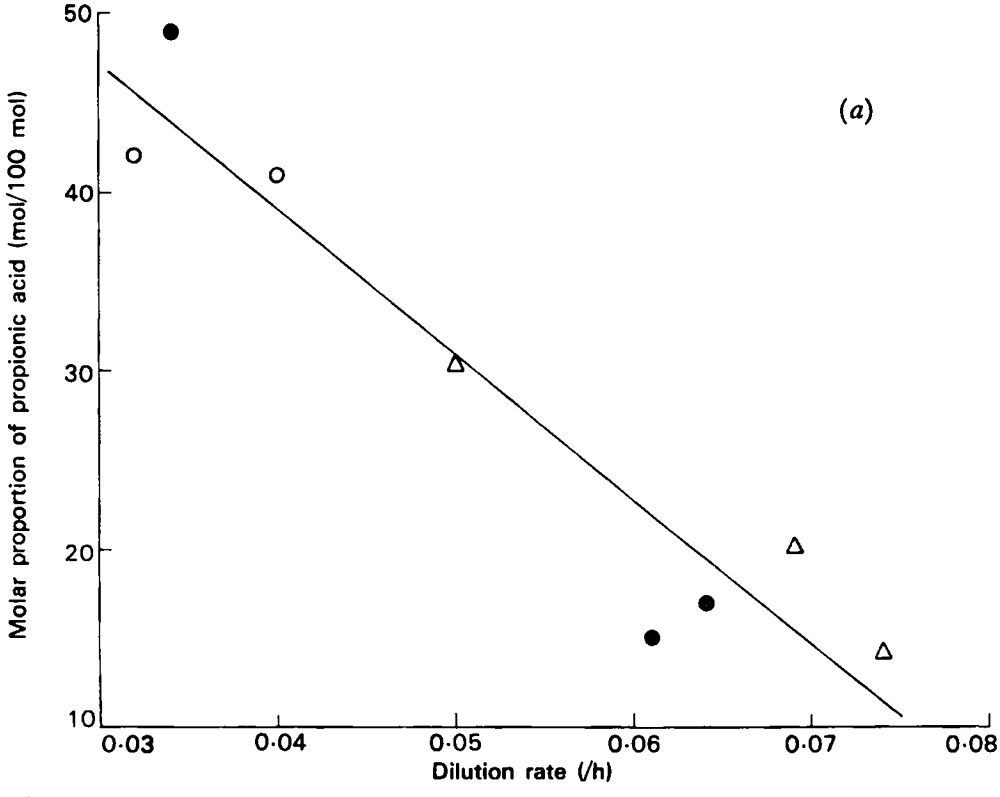
Bray, G. A. & York, D. A. (1971). *Physiol. Rev.* **51**, 598.
Du, J. T. & Kruger, F. A. (1972). *J. Nutr.* **102**, 1033.
Martin, R. J. (1974). *Life Sci.* **14**, 1447.
Uchiyama, M., Nakagawa, M. & Okui, S. (1967). *J. Biochem., Tokyo* **62**, 1.
Wahle, K. W. J. (1974). *Comp. Biochem. Physiol.* **48B**, 565.

The effect of altering dilution rate on the pattern of fermentation in the rumen. By D. J. THOMSON, D. E. BEEVER, D. C. MUNDELL, M. L. ELDERFIELD and D. G. HARRISON, *Grassland Research Institute, Hurley, Maidenhead, Berks. SL6 5LR*

It has previously been demonstrated with a chopped forage and cereal (10:1) diet (Harrison, Beever & Thomson, 1974), and with a flaked maize and pelleted dried grass (3:2) diet (Harrison, Beever, Thomson & Osbourn, 1975), that intraruminal infusion of artificial saliva increased dilution rate and lowered the proportion of propionic acid in the rumen liquor of sheep.

The mixed mineral salts of artificial saliva (McDougall, 1948) were incorporated in a diet of flaked maize and pelleted dried grass (3:2) at rates of 57 (diet A) and 114 (diet B) g/kg dry matter. Equivalent amounts of gross energy and nitrogen in the form of the control, unsupplemented diet (C), and diets A and B were fed hourly to lambs, each fitted with a rumen cannula, in a 3×3 Latin-square experiment. Dilution rate (Harrison, 1974), volatile fatty acid (VFA) production rate (Weller, Gray, Pilgrim & Jones, 1967) and faecal and urinary excretion of energy and N were measured at the end of each period of 50 d, during which VFA proportions were monitored at frequent intervals. Observations were not obtained on one lamb given the control diet due to incomplete consumption of food.

Dilution rate was negatively related to the molar proportion of propionic acid and positively related to the molar proportion of acetic acid (Fig. 1 *a* and *b*).



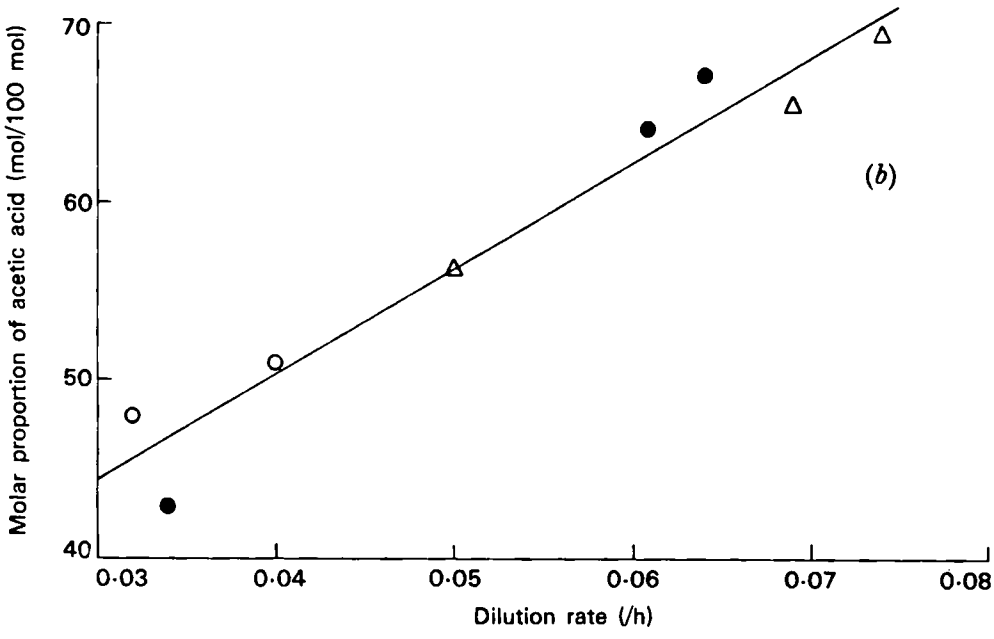


Fig. 1. Relationship between dilution rate and molar proportion of propionic acid (*a*) and molar proportion of acetic acid (*b*) in rumen liquor of lambs given the control, unsupplemented diet C (○), diet A (57 g mineral salts/kg), (●)- and diet B (114 g mineral salts/kg) (△).

Total VFA production rates were not significantly different for lambs given the three diets ($P<0.05$). The mean dilution rates (/h) and molar proportions of propionic and acetic acid (mol/100 mol) for the three diets were 0·036, 0·053 and 0·064; 44 (±1·5), 27 (±1·9) and 19 (±0·8); 46 (±1·2), 58 (±1·4) and 65 (±0·7) for diets C, A and B respectively. The apparent digestibility of N was lower ($P<0.05$), but N retention higher ($P<0.05$), for lambs given the mineral-supplemented diets compared with the control diet.

The increase in dilution rate and the alteration in the end-products of fermentation observed in this experiment were associated with changes in the microbial population of the lambs given the mineral-supplemented diets (Latham & Sharpe, 1975).

REFERENCES

Harrison, D. G. (1974). *Newsl. Applic. nucl. Meth. Biol. Agric.* **3**, 8.
Harrison, D. G., Beever, D. E., & Thomson, D. J. (1974). *Proc. Nutr. Soc.* **33**, 43A.
Harrison, D. G., Beever, D. E., Thomson, D. J. & Osbourn, D. F. (1975). *J. agric. Sci., Camb.* (In the Press.)
Latham, M. J. & Sharpe, M. E. (1975). *Proc. Nutr. Soc.* **34**, 113A.
McDougall, E. I. (1948). *Biochem. J.* **43**, 99.
Weller, R. A., Gray, F. V., Pilgrim, A. F. & Jones, G. B. (1967). *Aust. J. agric. Res.* **18**, 107.

Rumen microbial population of lambs given mineral-supplemented diets.

By M. J. LATHAM and M. ELISABETH SHARPE, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Using lambs in a 3×3 Latin-square experiment, Thomson, Beever, Mundell, Elderfield & Harrison (1975) have shown that the inclusion of mineral salts at levels of 57 (diet A) and 114 (diet B) g/kg dry matter in a flaked maize–pelleted, dried grass (60:40) diet (diet C) caused the rumen dilution rate to increase and reduced the molar proportion of propionate in the rumen fermentation. The rumen microflora of the lambs was examined from samples taken on two occasions during the last two periods at least 1 week after the fermentation had become stable. Direct total and differential microscopical counts and viable counts of total bacteria and bacterial types (Latham, Sharpe & Sutton, 1971) were determined.

In lambs with high-propionate fermentations (diet C), selenomonads and bacteroides predominated (Table 1). On giving diets A and B, bacteroides

Table 1. *Bacteria isolated from the rumens of lambs given control and mineral-supplemented diets*

Diet	Total no. of samples	Total no. of isolates	Types of bacteria (% total isolates)				
			Sels	Cocci	Bact	But	Others
Control	3*	244	54·5	9·0	27·9	2·5	6·1
57 g minerals/kg	4	286	30·8	47·9	2·4	4·5	14·4
114 g minerals/kg	4	304	52·0	35·2	6·9	2·6	3·3

Sels, selenomonads; Cocci, Gram-variable, mainly chain-forming cocci; Bact, bacteroides; But, butyrivibrio; Others, organisms of small numerical importance.

*Isolation medium of fourth sample overgrown with a motile organism.

decreased and Gram-variable, chain-forming cocci became the predominant organisms. Most probable numbers (MPN) of cellulolytic bacteria showed large between-animal variations with all diets. MPN of lactate-fermenting bacteria were reduced in animals on diet B. There were no consistent differences between diets in numbers of butyrivibrios or protozoa.

The large proportions of selenomonads and bacteroides associated with diet C are consistent with high-propionate fermentations. The role of the large numbers of chain-forming cocci (diets A and B) is not yet understood. In some respects these organisms resemble *Megasphaera elsdenii,* but the fermentation shift away from propionate suggests that these organisms were not involved in the conversion of lactic to propionic acid in vivo, a role frequently attributed to *Megasphaera.* In vitro nutritional tests suggest that the high levels of bicarbonate present in the mineral diets may have favoured the growth of these organisms in vivo.

REFERENCES

Latham, M. J., Sharpe, M. E. & Sutton, J. D. (1971). *J. appl. Bact.* **34**, 425.
Thomson, D. J., Beever, D. E., Mundell, D. C., Elderfield, M. L. & Harrison, D. G. (1975). *Proc. Nutr. Soc.* **34**, 111A.

The body density and total body potassium content of sixty-eight men and women of different ages and a variety of physiques. By J. WOMERSLEY, K. BODDY and J. V. G. A. DURNIN, *Institute of Physiology, The University of Glasgow and Scottish Research Reactor Centre, East Kilbride, Glasgow*

Groups of sedentary young men and women, physically active young men and women, young obese men and women, and older men and women were investigated. The density of each subject was measured by the technique of underwater weighing (Durnin & Rahaman, 1967). The total body potassium content of each subject was measured by the Merlin mobile whole-body radioactivity monitor (Boddy, King, Tothill & Strong, 1971).

Compared with the sedentary young groups, both the physically active and obese groups have a very much increased body K content, whereas the body K tends to decline in the older groups. When the fat-free mass (FFM) of each group of subjects is estimated from the density and K measurements, it is evident that the values which are commonly used for the density and K content of the FFM are not appropriate for all the groups. Possible adjustments to these values will be suggested for some of the groups of subjects, and the implications that these changes may have on the likely composition of the tissue which differentiates individuals in the different groups will be discussed.

REFERENCES

Boddy, K., King, P. C., Tothill, P. & Strong, J. A. (1971). *Physics Med. Biol.* **16**, 275.
Durnin, J. V. G. A. & Rahaman, M. M. (1967). *Br. J. Nutr.* **21**, 681.

The Ethiopian famine of 1973–4. 1. Wollo province. By J. SEAMAN and J. F. J. HOLT, *London Technical Group, c/o Institute of Biology, London SW7*

The famine which occurred in the province of Wollo in mid-1973 was the culmination of 6 years of declining rainfall in the region. A poor harvest from the long rains of 1972 was followed by complete failure of the short 1973 rains and this led to widespread starvation.

The effects of famine were not uniform, but were in general least in the western highland areas and most severe in the lower, eastern, agricultural areas and the Danakil desert. Within the agricultural population, starvation affected the poorest people, mostly tenant farmers and small landowners, because of their own limited food production and a twofold rise in the market price of cereals. At no time was grain unavailable in local markets and large amounts of cereals produced in the province were exported for sale in urban areas. Market prices collected by us showed that the price of cereals had returned to near pre-famine levels by October 1973. For a majority of the population the effects of the drought were felt through loss of wealth and the breaking up of families rather than through hunger.

An aerial survey (Anonymous, 1973) in late 1973 suggested that by then the only devastated area was a strip of poor-quality marginal agricultural land, some 10 km

wide and 100 km long, on the edge of the desert, that normally supported a sparse agricultural population.

Reports of large congregations of people in roadside towns suggest that the peak of the famine occurred between May and August 1973, and it declined rapidly during the near-normal 1973 long rains, leaving a residual population of about 15000 in thirteen roadside camps. The population of the province is estimated at 2·5 million people.

In March 1974 we conducted a survey in the Raya and Kobbo awraja of Wollo province prior to establishing a mother and child health programme for the 'Save the Children Fund'. At that time, and before large-scale food distribution had begun in the rural areas, nutritional status as gauged by weight and height measurements was unexceptional for an Ethiopian agricultural population. A single child with severe protein-energy malnutrition was found from the measured population of 618 children under 14 years of age. Death rates for the preceding year were established by interview of families in nine villages (Table 1).

Table 1. *Crude death rates in Raya and Kobbo awraja, Wollo Province, Ethiopia*

Age-group (years)	<1	1–4	5–9	10–14	15–44	45+
Crude death rate/1000 per year	212	214	97	13	40	130

This represents a crude death rate of 82 per 1000 people, although the small number of infants found may indicate that the mortality in this group was underestimated. A crude death rate of 20–30 per 1000 total population was found in north-western Ethiopia in 1969 (J. P. W. Rivers, personal communication). The excess number of deaths due to the drought in the province was therefore probably not less than 40000.

We are grateful to the Save the Children Fund, London, for financial support.

REFERENCE

Anonymous (1973). *A Preliminary Assessment of the Requirements for the Supplementary Supply of Seed, and Draft Oxen in the Drought-affected Areas of Tigre, Wollo and N. Shoa for the 1966 (E.C.) Crop Seasons.* Imperial Ethiopian Government Mimeo.

The Ethiopian famine of 1973–4. 2. Harerge province. By J. F. J. HOLT, J. SEAMAN and J. P. W. RIVERS*, *London Technical Group, c/o Institute of Biology, London SW7*

Despite wide publicity given to press reports of famine in Ethiopia from 1973 onwards, there is little factual information about what actually happened. In order to help rectify this situation we undertook for the Ethiopian Relief and Rehabilitation Commission a nutritional survey of Harerge province, south-east Ethiopia. The survey was conducted during May and June 1974 when it was believed there was widespread famine in the province.

*On leave of absence from the Nuffield Institute of Comparative Medicine, Zoological Society of London.

Clinical and anthropometric surveys of children of both pastoral and agricultural populations failed to reveal a current acute famine situation. Clinical protein-energy malnutrition was rarely seen. Nutritional status was poor but not exceptional for an Ethiopian population and certainly did not justify the widely-drawn parallel with the situation in the Biafran enclave.

However, reported mortality amongst under-fives during the previous year indicated that there had been a catastrophe in the area. Over half the sixty-five villages studied reported that more than 20% of this age group had died in the last year, and demographic observations confirmed this. The range of reported mortality from village to village was 0–87%. Villages with high mortality were widely scattered and no areas of consistently high mortality were found. However, it was evident that the pastoralists had, in general, experienced a higher mortality than agriculturalists. Both populations reported birth rates below the expected value, and pastoralists also reported that exceptionally large numbers of adults were absent from the village either with livestock or seeking work.

Both agriculturalists and pastoralists reported massive losses of livestock during the previous year. Consequently, the market price of animals had fallen dramatically in many areas, although by May 1974 it had virtually returned to pre-drought level.

The loss of livestock and the fall in their value had represented a substantial loss in purchasing power for the pastoralists, who usually sold animals in order to purchase cereals for their own staple diet. Their position had been further weakened by a continuous rise in cereal prices, which we found to be 2–3 times normal.

Thus, at the time of the survey the major effect of the drought was an economic one. Famine-related deaths and malnutrition were not at the time a problem, although a famine had probably occurred in the previous year. Food supply and nutritional status were precarious but not as catastrophic as was supposed, and such improvement as had occurred could not be accounted for by the limited spread of food aid.

The widespread misconception that a famine existed in May 1974 was probably due to reports originating from roadside clinics which, by their nature, tended to attract the destitute. Continuing reports of famine since October 1974 are difficult to evaluate for the same reason, and highlight the seriousness of the failure to organise surveillance schemes after this survey.

The survey was funded by UNICEF, USAID, Christian Aid and Oxfam (UK).

The effect of breast-feeding and artificial feeding on body-weights, skinfold measurements and food intakes of forty-two primiparous women. By D. J. NAISMITH and CAROLYN D. RITCHIE, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

Indirect measurements of the components of weight gain during pregnancy in women eating to satisfy their appetites suggest that approximately 4 kg fat is

stored (Hytten & Leitch, 1964). Analyses of the bodies of rats have revealed a cycle of fat retention during gestation followed by fat mobilization during lactation, the energy from fat being used to subsidize the high energy cost of lactation (Naismith, 1971). Evidence for such a cycle in human reproduction was sought in the present investigation.

Forty-two healthy primiparous women volunteered for the study. Their infants were born between 38 and 42 weeks of gestation, and weighed more than 2·5 kg at birth. Twenty-two agreed to feed their infants solely at the breast for 3 months; twenty bottle-fed their infants. Each mother was weighed in her home 10 d post partum, and four measurements of skinfold thickness (biceps, triceps, subscapular and supra-iliac) were taken for calculation of total body fat (Durnin & Rahaman, 1967). These measurements were repeated at fortnightly intervals for 12 weeks, and again at 6 months. Food intakes were estimated by the method of monthly recall, with a 3 d recorded food intake each month as a cross-check. Our findings were:

(Mean values with their standard errors)

Group	Weight gain during pregnancy (kg)	Change in body-weight (kg)		Daily energy intake over 3 months	
		At 3 months	At 6 months	(MJ)	(kcal)
Breast-feeding	13·2±0·75	−2·6±0·47*	−2·7±0·62*	12·0±0·44	2930±106
Bottle-feeding	13·9±0·83	−2·9±0·47*	−4·4±0·70*	8·5±0·52	2070±127

Difference from initial body-weight significant: *$P<0·0005$.

A significant decrease in the supra-iliac skinfold thickness was found (breast-feeding, −2·1 mm, $P<0·05$; bottle-feeding, −2·6 mm, $P<0·05$), but no consistent changes were observed at the other sites measured. Nevertheless, both groups showed considerable weight losses over the period of study. Eleven of the bottle-feeding mothers consciously restricted food intake to less than 8·2 MJ (2000 kcal)/d, whereas only one lactating mother, who admitted to dieting, consumed less than 10·3 MJ (2500 kcal)/d. The study has shown that breast-feeding does induce the catabolism of body fat, even when energy intakes exceed the recommended intake for lactation (11·3 MJ (2700 kcal)/d).

Two mothers who had gained only 4·5 and 5·0 kg during pregnancy persisted with breast-feeding for 8 weeks, but did not provide enough milk to satisfy their infants. Their mean daily food intakes during lactation were 12·0 MJ (2910 kcal) and 10·0 MJ (2420 kcal)/d respectively. This observation suggests that their failure to build up an energy reserve during pregnancy may not be unrelated to their poor lactational performance.

REFERENCES

Durnin, J. V. G. A. & Rahaman, M. M. (1967). *Br. J. Nutr.* **21**, 681.
Hytten, F. E. & Leitch, I. (1964). *The Physiology of Human Pregnancy.* Oxford: Blackwell.
Naismith, D. J. (1971). *Proc. Nutr. Soc.* **32**, 93A.

A comparison of growth in wholly breast-fed infants and in artificially fed infants. By CAROLYN D. RITCHIE and D. J. NAISMITH, *Department of Nutrition, Queen Elizabeth College, London W8 7AH*

A study was made of the growth of infants of the mothers described in the previous paper (Naismith & Ritchie, 1975). Twenty infants were fed solely at the breast for 3 months, and nineteen were artificially fed from birth, supplementary feeding being introduced when thought to be necessary by the mothers. Measurements of weight and length were made 10 d after birth, and at 14 d intervals thereafter for 12 weeks. Final measurements were made at 6 months. Accurate food intakes of the bottle-fed infants were recorded at the beginning of the study and for one week at the end of each month.

The breast-fed infants were, on average, 0·33 kg heavier and 10 mm longer than the bottle-fed infants at 10 d, but growth velocities (in weight and length) were found not to be related to starting values. The results are given in Table 1.

Table 1. *Mean velocities of growth in body-weight (g/d) and length (mm/d) in breast-fed and artificially-fed infants*

	Breast-fed (ten male, ten female)				Artificially-fed (nine male, ten female)			
Age ...	3 months		6 months		3 months		6 months	
	Weight	Length	Weight	Length	Weight	Length	Weight	Length
Males	29·4	11·0	22·7	8·5	33·3	12·6***	26·7***	9·7***
Females	26·1	10·5	20·4	8·1	29·9	11·8*	23·1**	9·2**
All infants	27·8	10·7	21·6	8·3	31·5**	12·2***	24·8***	9·4***

Value differs significantly from that for corresponding breast-fed infants: *$P<0.05$, **$P<0.025$, ***$P<0.01$.

No differences were found in the rate of increase in weight or length between the two groups within the first 6 weeks of life. During the second month, however, the bottle-fed infants began to grow faster both in length and weight, so that the mean velocities of growth over the first 3 months were significantly different. These differences persisted at 6 months. By the 6th week, eight of the artificially fed infants were receiving solids, but in one only did this make an appreciable contribution to the diet (11% of the total energy). At 14 weeks, ten were receiving between 10% and 40% of their energy from solids, but these foods were used to replace rather than to supplement the milk formulas. No correlation was found between the velocity of growth in length or in weight and daily food (energy) intake. The mean energy intake, measured at 3 months, was, however, only 464 kJ (111 kcal)/kg per d, i.e. substantially less than the recommended intake (502 kJ (120 kcal)/kg).

Our observations suggest that a high intake of protein (mean value 23·8 g/d) was the major factor in the aetiology of accelerated growth in the artificially fed infants.

REFERENCE

Naismith, D. J. & Ritchie, C. D. (1975). *Proc. Nutr. Soc.* **34**, 116A.

Nutrient intake of students in two different types of residences. By D. L. HAGGER (introduced by N. G. NORGAN), *Department of Human Sciences, University of Technology, Loughborough, Leicestershire*

A dietary and social survey was carried out at Loughborough University to investigate the nutritional and social implications of two different types of student residences. During a 7 d period in May, fifty-five students in self-catering flatlets, where they cooked for themselves, and fifty-four students in the traditional halls of residence, where all meals were provided, recorded all they ate and drank. Those in self-catering flatlets weighed all the items, while those in the halls of residence described the portion sizes they were eating and the author weighed standard servings from the hall kitchens.

The food and nutrient intakes were calculated by a computer program developed by Beechams Research Unit, based on the food tables of McCance & Widdowson (1969). The nutrient intake results are shown in Table 1 and the percentages of students receiving less than 90% of recommended intakes (Department of Health & Social Security, 1969) are given in parentheses.

Although the mean daily intakes of all four groups were above the recommended intakes, wide ranges were found. Most of the low intakes were recorded in the self-catering flatlets, where a number of students had low intakes of energy, nicotinic acid and thiamin. Of particular note was the high number of women in self-catering flatlets with low intakes of iron. These findings were similar to those of Stordy & Cowhig (1972) in their study of students at Surrey University.

Table 1. *Mean daily energy and nutrient intake of students in two different types of residence*

(Mean values and ranges: percentage with <90% of recommended intake in parentheses)

	No.	Energy (MJ)	Protein (g)	Iron (mg)	Thiamin (mg)	Riboflavin (mg)	Nicotinic acid (mg)	Ascorbic acid (mg)
Self-catering								
Male	38	12·0(26)	91(5)	17(0)	1·5(16)	2·4(5)	25(16)	63(8)
		6·4–17·8	54–135	11–24	0·7–3·7	1·2–3·6	9–56	22–150
Female	17	9·6(35)	67(0)	12(41)	1·3(12)	1·7(6)	20(36)	63(6)
		7·7–12	57–107	8–17	0·7–2·9	1·1–2·6	7–38	19–112
Halls of residence								
Male	35	13·4(0)	103(0)	17(0)	1·6(0)	2·1(9)	26(0)	64(0)
		10·8–17·6	69–136	10–25	1·2–2·2	1·2–3·5	19–42	29–125
Female	19	11·5(10)	93(0)	20(5)	1·4(0)	1·8(0)	21(5)	55(0)
		4·9–15·5	53–125	14–28	1·0–1·9	1·2–2·5	12–31	27–99

The students' expenditure patterns, examination performance, social involvement and their visits to the campus medical centre were of interest and will be described.

It was concluded that, although self-catering accommodation allowed the students a great deal of freedom in their life style and diets, it also made it easy for them to adopt an inferior diet to that of the students living in the traditional halls of residence.

REFERENCES

Department of Health & Social Security (1969). *Rep. publ. Hlth med. Subj., Lond.* no. 120.
McCance, R. A. & Widdowson, E. M. (1969). *Spec. Rep. Ser. med. Res. Coun.* no. 297.
Stordy, B. J. & Cowhig, J. R. (1972). *Proc. Nutr. Soc.* **31**, 81A.

Binding of calcium by dietary fibre: its relationship to unsubstituted uronic acids. By W. J. BRANCH, D. A. T. SOUTHGATE and W. P. T. JAMES, *Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ*

Previous work has suggested that fibre may limit the absorption of calcium and thus constitute a potential disadvantage to its introduction in subjects with marginal vitamin D deficiency (James, 1975). The non-cellulosic polysaccharides of the plant cell wall, not the crude fibre content, comprise the major fraction of dietary fibre (Southgate & Durnin, 1970), and in theory may be responsible for cation binding. The residues of samples extracted with dilute methanol (850 ml/l) and diethyl ether from twenty-five plant foods of known fibre content were assessed for Ca binding with isotopic techniques. Substantial pH-dependent binding was demonstrated for all samples, but the extent of binding varied. The pH-dependency suggested that the ionization of the carboxyl groups of uronic acids might be responsible, and a significant relationship between the uronic acid content and Ca binding was found ($P<0.001$). Some fibre residues bound less Ca than anticipated but were derived from foods known to have higher proportions of methyl-substituted uronic acids, which would be unavailable for Ca binding. Calculations suggest that a vegetarian diet as measured by Southgate & Durnin (1970) has sufficient uronic acid groups available within its non-cellulosic fraction to bind approximately 360 mg dietary Ca/d. Dietary phytate and oxalate would increase the amount of Ca bound.

Fractions of fibre unmeasurable by techniques used for crude fibre analysis would therefore seem capable of binding appreciable amounts of dietary Ca.

REFERENCES

James, W. P. T. (1975). *J. Coll. gen. Practnrs* (In the Press.)
Southgate, D. A. T. & Durnin, J. V. G. A. (1970). *Br. J. Nutr.* **24**, 517.

The possible effect of L-leucine on the nicotinic acid requirement of the rat. By JENNIFER A. MANSON, *Department of Applied Biology, University of Cambridge, Cambridge CB2 3DX*

The suggestions (Gopalan & Srikantia, 1960; Gopalan, 1969) that high leucine levels in diets are pellagragenic have renewed interest in the influence of amino acids on nicotinic acid requirements.

Manson & Carpenter (1974) recently reported results with dogs. We have now studied the nicotinic acid requirements of 21-d-old male rats of the CFY strain (Carworth Europe Ltd, Huntingdon). The basal diet was (g/kg): casein 60, gelatin 60, maize oil 50, L-threonine 1·5, L-cystine 4, minerals, vitamins (excepting nicotinic acid) and sucrose to 1000. This diet was estimated to contain 0·7 g tryptophan/kg and was calculated to be limiting in nicotinic acid and tryptophan.

It was found in a preliminary experiment that in the presence of 25 mg nicotinic acid/kg, 0·25 g L-tryptophan/kg supported maximal growth, but in the absence of nicotinic acid more than 0·6 g L-tryptophan/kg diet was required for comparable

growth. The basal diet with 0·25 g added L-tryptophan/kg was then used to measure the response to nicotinic acid in the presence and absence of an additional 15 g L-leucine/kg. The mean weight gains (g) per rat over 18 d on different treatments were:

	Nicotinic acid (mg/kg diet)				
Diet	0	2	7·5	15	25
Basal + tryptophan	5	11	33	56	64
Basal + tryptophan + leucine	11	13	30	28	30

Each value is a mean from four cages each containing two rats and has a pooled estimate of SE of 3·6 g (30 df).

The addition of 15 and 25 mg nicotinic acid/kg supported rapid growth in the absence of added leucine, but not in its presence. We then studied in a similar experiment whether the growth limitation in the presence of added leucine could be overcome with even higher levels of nicotinic acid or tryptophan. The mean weight gain and 'appetite quotient' (food consumption (g/d)/unit metabolic body-weight ($g^{0·75}$)) over 18 d with different dietary supplements were:

Nicotinic acid (mg/kg)		18	25	100	250	18	18
Tryptophan (g/kg):		0·25	0·25	0·25	0·25	1·25	2·25
Weight gain (g)	Without leucine	69	75	77	77	63	57
	With leucine	26	32	50	41	28	31
		Pooled SE of mean 5·6 (36 df)					
Appetite quotient	Without leucine	0·38	0·39	0·39	0·38	0·35	0·34
	With leucine	0·27	0·30	0·34	0·31	0·28	0·28
		Pooled SE mean 0·019 (36 df)					

It appears that leucine still limits growth regardless of the level of nicotinic acid or tryptophan included in the diet. In every instance, it reduces food intake as measured by the 'appetite quotient' (Carpenter, 1953).

Raghuramulu, Narasinga Rao & Gopalan (1965) did not observe a growth depression in young rats with 15 g L-leucine/kg added to a diet containing 110 g casein/kg. However, growth depressions with excess leucine have been reported (cf. review by Harper, Benevenga & Wohlhueter, 1970), and it has been concluded that such an amino acid imbalance does have the first effect of reducing voluntary food intake.

We conclude that even under conditions where addition of leucine has depressed the growth rate of young rats, it has not done so by increasing the requirement for nicotinic acid or tryptophan.

REFERENCES

Carpenter, K. J. (1953). *J. Nutr.* **51**, 435.
Gopalan, C. (1969). *Lancet* i, 197.
Gopalan, C. & Srikantia, S. G. (1960). *Lancet* i, 954.
Harper, A. E., Benevenga, N. J. & Wohlhueter, R. M. (1970). *Physiol. Rev.* **50**, 428.
Manson, J. A. & Carpenter, K. J. (1974). *Proc. Nutr. Soc.* **33**, 103A.
Raghuramulu, N., Narasinga Rao, B. S. & Gopalan, C. (1965). *J. Nutr.* **86**, 100.

The effect of some antioxidants on lipofuscin accumulation in rat brain.

By D. N. RUDRA, J. W. T. DICKERSON and R. WALKER, *Department of Biochemistry, University of Surrey, Guildford GU2 5XH,* and J. CHAYEN, *Mathilda and Terence Kennedy Institute of Rheumatology, Hammersmith, London W6 7DW*

Lipofuscin is formed by autoxidation of polyunsaturated fatty acids and accumulates with age, particularly in post-mitotic tissue such as heart (Strehler, Mark, Mildan & Gee, 1959; Munnell & Getty, 1968) and brain (Reichel, Hollander, Clarke & Strehler, 1968). There is evidence that lipofuscin accumulates more rapidly in vitamin E deficiency (Mason, Dam & Granados, 1946) and that autoxidation can be retarded by dietary supplementation with vitamin E (Weglicki, Reichel & Nair, 1968). Vitamin E-deficient rats showed a greater loss of memory function with age than did controls (Lal, Pogacar, Daly & Puri, 1973) and the oxidation processes leading to lipofuscin accumulation may play a major role in cell ageing and senescence (Strehler & Barrows, 1970). The effects of long-term dietary supplementation with α-tocopherol or a synthetic antioxidant, ethoxyquin, on lipofuscin accumulation in rat brain have therefore been studied.

Three experimental groups of male Wistar rats were maintained from weaning on a commercial powdered diet (Spillers small animal diet) containing 25 g antioxidant-free arachis oil/kg and supplemented with (g/kg): 0·5 DL-α-tocopheryl acetate, 5 DL-α-tocopheryl acetate and 5 ethoxyquin respectively. Pair-fed control groups were maintained concurrently on non-supplemented diets. Animals receiving α-tocopheryl acetate were reared for 702 d and those receiving ethoxyquin for 557 d. In the latter instance, nephrotoxicity of ethoxyquin led to the curtailment of the experiment (Rudra, Dickerson & Walker, 1974). At the end of the experimental period the rats were decapitated and the brain was quickly removed and cut in half longitudinally. Lipofuscin was assayed on 5 μm sections by fluorescence microscopy and microdensitometry.

Supplementation with antioxidants had no significant effect on the number of cells containing lipofuscin, but the amount present as estimated by the intensity of fluorescence was significantly lower in all experimental groups than in pair-fed controls. The amount of lipofuscin was slightly lower in the brain of animals receiving 5 g than those receiving 0·5 g α-tocopheryl acetate/kg.

These results will be discussed in relation to the possible advantages of supplementary vitamin E at levels higher than those required to prevent frank deficiency in the rat.

We thank Associated Health Foods for financial support.

REFERENCES

Lal, H., Pogacar, S., Daly, P. R. & Puri, S. K. (1973). *Prog. Brain Res.* **40**, 129.
Mason, K. E., Dam, H. & Granados, H. (1946). *Anat. Rec.* **94**, 265.
Munnell, J. F. & Getty, R. (1968). *J. Geront.* **23**, 154.
Reichel, W., Hollander, J., Clark, J. H. & Strehler, B. L. (1968). *J. Geront.* **23**, 71.
Rudra, D. N., Dickerson, J. W. T. & Walker, R. (1974). *J. Sci. Fd Agric.* **25**, 1049.
Strehler, B. L. & Barrows, C. H. (1970). In *Cell Differentiation*, p. 266 [O. A. Schjeide and J. de Vellis, editors]. New York: Van Nostrand-Reinhold.
Strehler, B. L., Mark, D. D., Mildan, A. S. & Gee, M. V. (1959). *J. Geront.* **14**, 430.
Weglicki, W. B., Reichel, W. & Nair, P. P. (1968). *J. Geront.* **23**, 469.

The effect of fenfluramine on blood glucose and ascorbic acid in normal subjects. By MAURICE GREENE and C. W. M. WILSON, *Department of Pharmacology, University of Dublin, Trinity College, Dublin, Eire*

Fenfluramine reduces tissue ascorbic acid (AA) concentrations in guinea-pigs, and administration of AA concurrently with fenfluramine inhibits its anti-obesity action (Odumosu & Wilson, 1973). Wilson (1974) suggested that this anti-obesity action of fenfluramine could be mediated through an effect on AA metabolism in obese subjects. In order to investigate the effect of fenfluramine, the drug (as Ponderax, 40 mg daily) was administered to twenty normal young adults for 2 weeks. Plasma leucocyte and lingual AA levels and blood glucose were monitored, initially and 3, 7 and 14 d after beginning treatment. Intake of AA was not altered during the period of the investigation from the pre-trial diet. Blood AA concentrations were measured by the method of Loh & Wilson (1971). Lingual AA was assayed by the chloroindophenol method (Cheraskin & Ringsdorf, 1968). The concentration of AA was measured in the morning specimens of urine on the specified days.

Plasma and leucocyte AA levels were not significantly altered after 14 d. Lingual AA decreased progressively during the 2 weeks, indicating tissue depletion of AA. A significant decrease had taken place by day 14 ($P<0.05$). Urinary AA concentration increased during the period of drug administration. Plasma glucose rose significantly even after 3 d of treatment ($P<0.01$) and remained significantly elevated until day 14. This contrasts with the observation of Butterfield & Whichelow (1968), who found that venous glucose was reduced in comparison with arterial glucose after fenfluramine administration.

Leucocytes were incubated with AA (30 mg/l) and AA+ fenfluramine (0·6 and 0·3 mg/l), using the method described previously (Loh & Wilson, 1970). No decrease in AA uptake took place in the presence of fenfluramine, indicating that cellular absorption of AA was unaffected. A fall in leucocyte AA level was observed in six subjects on day 3. After this initial decrease leucocyte AA values returned to normal by day 7.

It appears that this relatively low dose of fenfluramine affects blood glucose and some aspects of AA metabolism. Lingual AA is significantly reduced: urinary AA concentration is increased. Tissue AA depletion occurs in guinea-pigs, but this effect was not evident in all these normal subjects. However, leucocyte AA was decreased in some individuals.

Although concurrent alterations occur in blood glucose and AA levels following administration of fenfluramine to normal individuals, the implications of these changes are not apparent in normal individuals. The results are probably affected by intra-individual variations in tissue AA and glucose concentrations. These have a wide range in normal subjects and can buffer changes induced by drugs unless large doses are administered.

REFERENCES

Butterfield, W. J. H. & Whichelow, M. J. (1968). *Lancet* ii, 109.
Cheraskin, E. & Ringsdorf, W. M. (1968). *Int. Z. VitamForsch.* **38**, 114.

Loh, H. S. & Wilson, C. W. M. (1970). *Br. J. Pharmac.* **40**, 169P.
Loh, H. S. & Wilson, C. W. M. (1971). *Int. Z. VitamForsch.* **41**, 90.
Odumosu, A. & Wilson, C. W. M. (1973). *Br. J. Pharmac.* **48**, 326P.
Wilson, C. W. M. (1974). *Practitioner* **212**, 481.

The effect of dietary sucrose on lipid metabolism in Dutch belted rabbits (*Oryctolagus cuniculus*). By NANCY ANN WORCESTER and K. R. BRUCKDORFER, *Department of Biochemistry and Chemistry, Royal Free Hospital Medical School, London WC1*, and JOHN YUDKIN, *Servier Research Institute, Horsenden Lane South, Greenford, Middlesex*

Previous work comparing the atherogenic properties of sucrose and starch in the rabbit has been inconclusive (Kritchevsky & Tepper, 1967; Kritchevsky, Tepper & Kitagawa, 1973). We have compared the effects of the two carbohydrates on several indicators of atherogenesis. To minimize interaction with other dietary components, the rabbits were given a low-fat diet free from cholesterol.

Twenty-two young female Dutch belted rabbits (*Oryctolagus cuniculus*), each weighing about 1200 g, were divided into two groups, and given purified diets containing 560 g sucrose or maize starch/kg. This purified diet, mixed with decreasing proportions of ground stock diet, was introduced to the rabbits over a 30 d period. The rabbits were then given an 80% purified diet for a further 26 d.

There was no difference in total body-weights, or in the weights of the kidneys, spleens or adrenal glands in the two groups. However, liver weights were greater in the sucrose group.

The activity of hepatic fatty acid synthetase was not different on the two diets.

Blood samples taken after 33 and 56 d of feeding showed no difference in the plasma lipids.

Visual and histological examination revealed no atherosclerosis in the aortas of any of the rabbits. Nor was there any difference between the two dietary groups in the concentrations of lipids in the intima-media layer of the aortas.

These results contrast with the atherogenic effect of sucrose which has been found in other species. Plasma lipids are elevated by sucrose feeding in the rat (Bruckdorfer, Khan & Yudkin, 1971), spiny mouse (Bruckdorfer, Worcester & Yudkin, 1974), pig (Brooks, Miyahara, Huck & Ishizaki, 1972) and monkey (Lang & Barthel, 1972). Fatty acid synthetase activity is increased by sucrose feeding in the rat (Bruckdorfer *et al.* 1971) and chicken (Bruckdorfer, Kari-Kari, Khan & Yudkin, 1972), and in the spiny mouse, albino mouse and guinea-pig (Bruckdorfer, unpublished results).

REFERENCES

Brooks, C. C., Miyahara, A. Y., Huck, D. W. & Ishizaki, S. M. (1972). *J. Anim. Sci.* **35**, 31.
Bruckdorfer, K. R., Kari-Kari, B. P. B., Khan, I. H. & Yudkin, J. (1972). *Nutr. Metab.* **14**, 228.
Bruckdorfer, K. R., Khan, I. H. & Yudkin, J. (1971). *Biochem. J.* **123**, 7P.
Bruckdorfer, K. R., Worcester, N. A. & Yudkin, J. (1974). *Proc. Nutr. Soc.* **33**, 4A.
Kritchevsky, D. & Tepper, S. A. (1967). *Circulation* **36**, Suppl. 2, 22.
Kritchevsky, D., Tepper, S. A. & Kitagawa, M. (1973). *Nutr. Rep. int.* **7**, 193.
Lang, C. M. & Barthel, C. H. (1972). *Am. J. clin. Nutr.* **25**, 470.

The effect of dietary sucrose and hydrogenated coconut oil on atherosclerosis in Dutch belted rabbits (*Oryctolagus cuniculus*). By NANCY ANN WORCESTER and K. R. BRUCKDORFER, *Department of Biochemistry and Chemistry, Royal Free Hospital Medical School, London WC1*, and JOHN YUDKIN, *Servier Research Institute, Horsenden Lane South, Greenford, Middlesex*

There is conflicting evidence available on the atherogenic properties of both sucrose (Worcester, Bruckdorfer & Yudkin, 1975) and fats in rabbits. Kritchevsky, Moyer, Tesar, Logan, Brown, Davies & Cox (1954) reported that diets with saturated fat produced more severe atherosclerosis than did diets with polyunsaturated fats. Lambert, Miller, Olsen & Frost (1958), however, found a negligible difference in the atherogenic properties of saturated and polyunsaturated fat in diets with cholesterol.

Thirty-two young female Dutch belted rabbits (*Oryctolagus cuniculus*) were divided into three dietary groups. Two groups were given diets with sucrose. One of these diets contained a saturated fat (hydrogenated coconut oil (HCO)), the other a polyunsaturated fat (maize oil). A further group received a starch diet containing the HCO. All purified diets contained 480 g carbohydrate and 110 g fat/kg. Rabbits were gradually introduced to the purified diet over a 3·5 week period, then were given 80% purified diet for a further 19 weeks. Dietary cholesterol was increased from 0·5 to 1·9 g/kg during the experiment.

Body-weight was not influenced by the type of dietary carbohydrate. However, rabbits given HCO grew much slower than those given maize oil.

Fasted blood samples were taken after 6·5, 14 and 22 weeks of feeding. On the first occasion, plasma cholesterol concentration was elevated in the group given sucrose compared with starch, but not on the other two occasions; triglycerides were not different on any occasion. The first sample exhibited no effect of fat on plasma lipids; in the second sample, cholesterol concentration was higher in the HCO groups and in the third sample, the concentrations of both cholesterol and triglyceride were higher in the HCO groups.

All three dietary groups exhibited a wide range of aortic atherosclerosis. There was no difference in abdominal score, thoracic score, or area of sudanophilia between the sucrose and starch groups. However, animals given HCO showed more atherosclerosis ($P<0·02$) than did those given maize oil, as judged by all three tests.

The difference in individual responses of the rabbit, chicken (Worcester, Bruckdorfer & Yudkin, 1975) and man (Szanto & Yudkin, 1969) to sucrose feeding suggests that sucrose may be an atherogenic agent in some individuals but not others.

REFERENCES

Kritchevsky, D., Moyer, A. W., Tesar, W. C., Logan, J. B., Brown, R. A., Davies, M. C. & Cox, H. R. (1954). *Am. J. Physiol.* **178**, 30.
Lambert, G. F., Miller, J. P., Olson, R. T. & Frost, D. V. (1958). *Proc. Soc. exp. Biol. Med.* **97**, 544.
Szanto, S. & Yudkin, J. (1969). *Post-grad. med. J.* **45**, 602.
Worcester, N. A., Bruckdorfer, K. R. & Yudkin, J. (1975). *Proc. Nutr. Soc.* **34**, 124A.