The Two Hundred and Sixty-eighth Scientific Meeting of the Nutrition Society was held in the Barnes Hall, Royal Society of Medicine, Wimpole Street, London W1M 8AE on Friday, 17 May 1974, at 10.30 hours, when the following papers were read :

A dietary survey of some forge workers. By J. D. BROOKE, *Human Performance Laboratory, Physical Education Section, University of Salford* and L. F. GREEN, *Beecham Products Research Department, Randalls Road, Leatherhead, Surrey*

Accident rates among workers in the hot shops of a forge were highest between 08.00 and 10.00 hours, when little carbohydrate was being metabolized (Brooke, Toogood, Green & Bagley, 1973). A glucose syrup supplement given over 4 months reduced this rate (Brooke, Toogood, Green & Bagley, 1974).

During the research, two dietary surveys were made. Single day intakes were reported by ten workers once during each of 4 consecutive months, and by forty-four workers once at the end of experimentation. The day's intake was recorded for the periods: waking to 10.00 hours (1); 10.00 to 12.00 hours (2); 12.00 to 15.00 hours (3); 15.00 to 17.00 hours (4); and 17.00 hours to sleep (5). The energy contributions from fat, protein, carbohydrate and alcohol were computed (McCance & Widdowson, 1969).

The two surveys produced similar results. The histogram for the ten subjects (Fig. 1) shows marked asymmetry of intake, total energy and carbohydrate over the
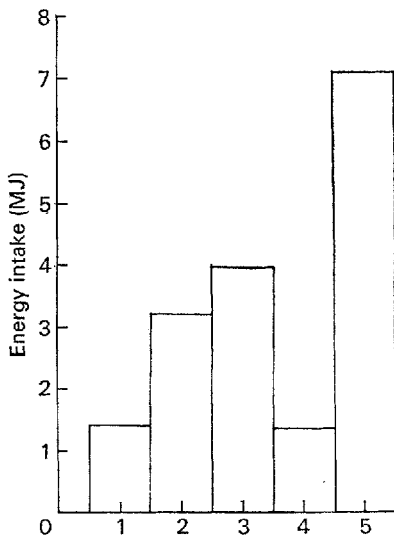

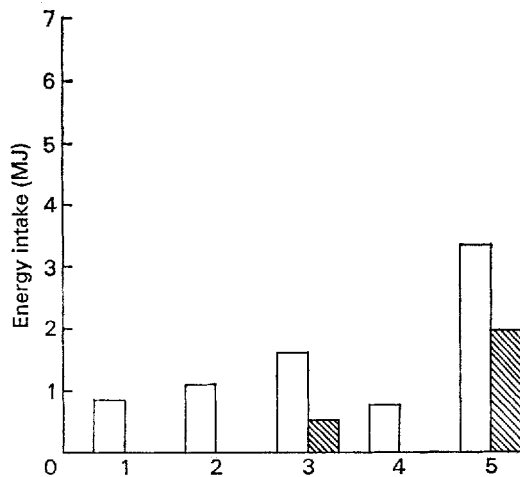Fig. 1. Mean daily energy intake during five periods of the day.

Fig. 2. Mean daily intakes of carbohydrate and alcohol during five periods of the day. □, carbohydrate; ▨, alcohol.

working day, with little ingested early morning. Daily proportions of carbohydrate and alcohol, and the subjects' mean body-weight, remained comparatively constant over 4 months; for the group of ten, alcohol contributed about 12% of total energy intake (Fig. 2).

The survey work of Miss S. Toogood, BSc and the co-operation of the forge personnel are gratefully acknowledged.

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Effect of ageing on fat mobilization in man. By G. L. S. PAWAN, *Metabolic Division, Department of Medicine, The Middlesex Hospital Medical School, London W1P 7PN*

Normal male volunteers in the following age groups: 18–29, 30–40, 41–50, 51–60, and 61–72 years (twenty men/group, all within 15% of ideal body-weight, and without a history of obesity or diabetes) were fasted for 24 h. Heparinized blood samples were drawn at 12 h and 18 h of the fast, and the total 24 h urine was obtained from each subject. The blood samples were immediately centrifuged, and the plasma was analysed for free fatty acids, free glycerol, and 'ketones' as described previously (Pawan, 1969*a*). The urine samples were assayed for fat-mobilizing substance (FMS) activity (Pawan, 1969*b*) after extraction as described by Kekwick & Pawan (1967). Lean body mass and fat mass of the subjects were determined from measurements of total body water by the ethanol method (Pawan & Hoult, 1963). Skinfold thickness measurements were made at the triceps, subscapular and supra-iliac sites in all subjects. The more important results are shown in Table 1.

Table 1. *Effect of ageing on plasma lipids, urinary fat-mobilizing substance (FMS) and body fat, measured after an 18 h fast, in men*

(Mean values with their standard errors for groups of twenty men)

	Men aged 18–29 years	Men aged 61–72 years
Free fatty acids (mmol/l)	1·85±0·3	0·95±0·2
Glycerol (mg/l)	26±5	15±3
'Ketones' (mg/l)	19·5±2	13±2
Urinary FMS activity	142±16	88±10
% Body fat	18·5±1·5	26·5±1·5

With increasing age, normal men exhibit an increase in the percentage of body fat and a decrease in lean body mass. This appears to be associated with a reduced ability to mobilize fat in response to the physiological stimulus of fasting. In older men, skinfold thickness measurements at the supra-iliac and subscapular sites are better indicators of total body fat than measurements at the triceps site.

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A comparison of the fatty acid composition of adipose tissue triglyceride from grass–fed and intensively–reared lambs. By J. Pearce, *Agricultural and Food Chemistry Research Division, Department of Agriculture, Newforge Lane, Belfast BT9 5PX and* D. M. B. Chestnutt, *Agricultural Research Institute of Northern Ireland, Hillsborough, County Down, Northern Ireland* (introduced by J. R. Todd)

Producers of intensively-reared, early-weaned lambs are experiencing difficulties in relation to the acceptability of carcases owing to an undesirable softness of the fat. In particular it has been observed that the subcutaneous adipose tissue is much softer than in traditionally-reared, grass-fed animals. This experiment was therefore conducted to compare the fatty acid composition of subcutaneous lipid from grass-fed and intensively-fed lambs.

Three lambs were weaned at 28 d of age onto a diet containing 850 g ground barley and 150 g soya-bean meal/kg (a mineral–vitamin supplement was also included) and were slaughtered at 37 kg live weight. Another three lambs, born around the same time, remained with their ewes and were also slaughtered at the same live weight; these traditionally-reared lambs were weaned normally onto grass. After slaughter, subcutaneous adipose tissue samples were taken from the loin region. The lipid was extracted with chloroform–methanol (2:1, v/v) (Folch, Lees & Sloane Stanley, 1957) and the triglyceride fatty acids methylated with 2 M-sodium methoxide in dry methanol. The resulting esters were separated by gas–liquid chromatography using columns packed with 6% diethyleneglycol succinate and 7% Apiezon L grease.

In general, the adipose tissue from the grass-fed animals contained more saturated fatty acids than that from intensively-reared lambs (Table 1); there was a greater

Table 1. *Composition of subcutaneous adipose tissue trigylceride from grass-fed and intensively-reared lambs ; fatty acids expressed as percentage of total fatty acids*

Fatty acid	Grass-fed lambs			Intensively-reared lambs		
	1	2	3	1	2	3
14:0	7·0	9·2	6·7	2·2	3·3	3·0
15:0	0·9	0·9	0·9	1·7	1·1	1·1
16:0	22·6	24·4	21·1	22·6	20·1	19·9
16:1	2·5	1·8	2·2	2·0	1·3	1·0
17:0	1·3	1·2	1·3	5·6	4·8	4·6
17:1	0·8	0·9	0·8	2·5	2·0	2·4
18:0	16·4	12·8	17·2	7·3	14·6	12·6
18:1	39·9	40·3	40·2	43·1	40·9	45·7
18:2	2·7	2·4	3·2	3·5	5·2	2·8
18:3	2·3	2·6	2·7	0·3	0·7	0·4
Branched-chain (monomethyl substituted C_{15}, C_{16}, C_{17} & C_{18} acids)	2·4	2·8	3·0	8·5	5·4	6·2

proportion of myristic acid (14:0) and stearic acid (18:0) in the grass-fed animals. Conversely, there was more oleic acid (18:1) and odd-numbered n-acids (15:0 and 17:0) in the intensively-reared lambs. There was also a greater amount of some unidentified fatty acids in the tissue from intensively-reared animals; these may include branched-chain acids as described by Garton, Hovell & Duncan (1972).

The differences we observed in the fatty acid composition of adipose tissue between grass-fed and intensively-reared lambs would account for the carcase characteristics of these animals. The softer fat in the intensively-fed animals may also be partly due to the production of odd numbered n-acids and branched-chain acids resulting from the increased availability of propionate from the rumen fermentation of cereal-based diets (Duncan, Ørskov & Garton, 1972).

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Rate of production of volatile fatty acids in the rumen of milking cows.
By J. D. SUTTON and E. SCHULLER, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

Volatile fatty acids (VFA) are the principal source of energy absorbed from the digestive tract of the ruminant. Isotope dilution techniques have been applied widely to the measurement of VFA production in the rumen of sheep, but only very rarely in dairy cows, and then with widely divergent results.

Three milking Friesian cows were each given a 'normal' ration of 5·5 kg hay and 10·8 kg dairy concentrates (180 MJ digestible energy (DE)) daily. They were also given a low-hay ration consisting of (kg/d): 1·0 hay, 5·5 dairy concentrate and 5·5 flaked maize (145 MJ DE) for cows A and B and 1·6 hay and 13·9 dairy concentrate (180 MJ DE) for cow C. All rations were offered at two frequencies, two and twenty-four times daily.

On two occasions for each cow on each treatment, 0·5–1·0 mCi [1-^{14}C]acetic acid was infused into the rumen for 22 h and samples were taken from four sites at frequent intervals during the last 12 h (Sutton, Macleod, Sissons & Johnson, 1972). The net production of total VFA was determined from the dilution of the infused tracer by the total VFA in a bulk sample covering the 12 h sampling period.

Table 1. *Rates of production of volatile fatty acids in relation to the digestible energy of the rations (J/kJ digestible energy)*

Ration	Feeds per d	Cow A	B	C	Mean	SE
Normal	24	509	493	441	481	21
	2	368	429	471	423	30
Low-hay	24	509	649	499	552	48
	2	399	471	396	422	25

The energy available from the VFA was calculated on the assumption that the relative net rate of production of the acids was the same as the relative proportions of the acids in the rumen contents.

There were no consistent differences in VFA production due to ration composition. With frequent feeding, rumen VFA provided about half the DE, results similar to those found for sheep (Leng & Murray, 1972). However, when rations were given twice daily, the estimated contribution of VFA to DE was on average about 18% less than with frequent feeding, although over-all digestibility was unaffected. No such difference due to feeding frequency was found for sheep given roughages (Gray, Weller, Pilgrim & Jones, 1967). Studies are now in hand to determine whether the difference in the present experiments is a real one or whether the use of isotope dilution procedures with twice-daily feeding leads to erroneous results because of the serious deviations from steady-state conditions.

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Absorption of maize oil infused into the duodenum of the sheep. By F. A. HARRISON, W. M. F. LEAT and A. FORSTER, *ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT*

In sheep under normal husbandry conditions most of the dietary esterified lipids are hydrolysed in the rumen and little triglyceride reaches the small intestine (see Garton, 1967). With the present trend of protecting dietary components, including lipid, from rumen degradation (e.g. Scott, Cook & Mills, 1971) it is important to obtain further information on the fate of triglycerides in the small intestine of the ruminant.

When maize oil (52·6% linoleic acid) was infused continuously at 2 g/h through a duodenal cannula of a sheep prepared with fistulation of the thoracic duct, there was a rapid change in the fatty acid composition of lymph triglycerides. Within 6 h the linoleic acid content of lymph triglycerides had increased from 6·6% to 45·9% whereas in phospholipids the increase was only from 26·8% to 32·5%. After 48 h of maize-oil infusion, the linoleic acid content of lymph phospholipids rose to 44·9%. Under control conditions the linoleic acid of lymph was transported equally by triglycerides and phospholipid; after infusion of maize oil for 48 h the major part of the lymph linoleic acid was transported by lymph triglycerides (Table 1, cf. Heath, Adams & Morris, 1964).

In control lymph 72·6% of the lipids were located in the very-low-density lipoprotein fraction and 27·4% in the chylomicron fraction, as defined by the method of Hatch & Lees (1968). However, after 24 h of maize-oil infusion, 38·5% of the lymph lipids were located in the very-low-density lipoprotein and 61·5% in the chylomicron fraction.

Table 1. *Fatty acids transported in the thoracic duct lymph of sheep before and 48 h after a continuous infusion of maize oil at 2 g/h (6 h collection period)*

	Transported in lymph (mg/h)						Fatty acids infused (mg/h)
	Control (A)		Maize-oil infusion (B)		Difference (B-A)		
Fatty acid	Trigly-cerides	Phospho-lipids	Trigly-cerides	Phospho-lipids	Trigly-cerides	Phospho-lipids	
16:0	112·5	17·6	195·4	23·4	+ 82·9	+ 5·8	278
18:0	174·0	20·1	183·1	40·8	+ 9·1	+20·7	52
18:1	69·1	20·1	304·7	16·8	+235·6	− 3·3	600
18:2	19·7	20·6	528·5	78·4	+508·8	+57·8	1052
18:3	8·5	6·8	21·0	4·8	+ 12·5	− 2·0	30
Total	473·9	103·8	1369·0	179·3	+895·1	+75·5	

To compare the absorption of the free fatty acids of digesta with the absorption of the continuously infused triglyceride, 2 g maize oil containing 10 μCi [1-^{14}C]-triolein was injected into the duodenum followed immediately by 100 μCi [^{3}H]-oleic acid. There was a distinct separation of the two isotopes in lymph with the maximum absorption of ^{3}H-labelled fatty acids occurring 1·25 h after injection compared with 2·25 h for ^{14}C-labelled fatty acids. The delay in the absorption of triglyceride compared with free fatty acids is probably a consequence of the unfavourable conditions for the hydrolysis of triglycerides by pancreatic lipase in the upper intestine of the sheep (Arienti, Harrison & Leat, 1974).

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Quantitative variations in the absorption of glucose during digestion of maize starch in the pig. By A. RERAT, A. AUMAITRE, P. VAUGELADE and P. VAISSADE, *I.N.R.A.—C.N.R.Z., 78350 Jouy-en-Josas, France.*

The study of the intestinal absorption of a given nutrient is facilitated by simultaneous measuring of the increase in its level in the efferent blood of the intestine (by the difference between portal and systemic concentrations) and of the blood flow rate in the portal vein (Rerat, 1971a, b). This procedure was applied to the study of the kinetics of carbohydrate absorption after ingestion of maize starch in the pig.

Four pigs (30–40 kg live weight) were fitted with permanent cannulas in the portal vein and the anterior vena cava, as well as with a probe connected to an electromagnetic flowmeter. From 10–14 d after the surgical operation, the animals received, every 2–3 d, an experimental diet: a basal ration containing proteins, vitamins and minerals (150 g), and maize starch (400, 800, 1100 or 1200 g). This diet was offered after fasting periods of different lengths (16 or 22 h).

The amount of carbohydrate absorbed during a postprandial period of 8 h was calculated according to a previously described method (Rerat & Aumaitre, 1971).

For the same amount of starch eaten (400 g), the quantity of carbohydrates absorbed during the postprandial period (8 h) decreased by more than 100 g when the length of the preprandial fasting increased from 16 to 22 h. Thus, 16 h after a starch meal, the digestion of this carbohydrate is not completely finished: the products of this digestion are added to those of the experimental meal, giving an apparent absorption coefficient of about 120%. These facts are confirmed by blood concentration results: after 16 h of fasting, the portal glucose level is much higher than the peripheral one (difference of about 130 mg/l) whereas this is no longer the situation after 22 h of fasting (20 mg/l, mean from nine replicates).

After 22 h of fasting, small residues of the previous meal seem to remain, resulting in the absorption of a very small amount of carbohydrate during 8 h (22 to 30 h of fasting).

Expressed in absolute values, the quantity of carbohydrate absorbed increases with the quantity ingested; the increase in the amount absorbed becomes noticeable only about 4 h after the start of the meal.

Conversely, expressed in relative values, the proportion of carbohydrate absorbed decreases as the amount ingested is raised. Above an intake of 800 g carbohydrate, however, no further decline in the proportion absorbed occurs.

Giving the nitrogenous fraction of the meal alone results in an appreciable absorption of carbohydrate, which may be derived from the carbohydrate component of the protein concentrate and from the intestinal residues of the meal supplied 22 h earlier.

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Nicotinic acid deficiency in dogs with and without supplementary leucine in their diets. By JENNIFER A. MANSON and K. J. CARPENTER, *Department of Applied Biology, University of Cambridge, Cambridge CB2 3DX*

The suggestions (Gopalan & Srikantia, 1960) that high leucine levels in diets are pellagragenic and that isoleucine counteracts this effect (Krishnaswamy & Gopalan, 1971) has renewed interest in the influence of amino acids on niacin requirements. Neither we, nor Allen, Baker & Graber (1971), were able to precipitate nicotinic acid deficiency in chicks with leucine supplements. We therefore followed a published experiment with dogs (Belavady, Madhavan & Gopalan, 1967).

Beagle puppies (8 weeks old) were randomized onto three diets (1–3). They consisted of (g/kg): casein 180, cottonseed oil 110, minerals, vitamins (excepting nicotinic acid) with or without L-amino acid supplements (as shown in the table)

and maize starch to 1000. Each was mixed with half its weight of water, canned and lightly autoclaved to gel the starch. The puppies were housed in groups of four (from different treatments) but fed individually twice daily to a standard scale ((US) National Research Council, 1962) and given nicotinic acid by mouth.

After 75 d the puppies on all three diets were still gaining weight and looking healthy. The supplementary nicotinic acid was therefore halved. During the next month most lost appetite and body-weight, some showing hyperaemia in the mouth. Four (two on diet 1 and one each on diets 2 and 3) died suddenly, with appearance of heart failure at post mortem. The others showing severe weight loss were given nicotinic acid injections and quickly responded. The animals that died and those given injections are counted in the 'no. deficient':

		No. deficient/no. on diet		
Time from start of experiment (d)	Dosage of nicotinic acid (μg/kg body-wt per d)	1 (no supplement)	2 (12 g leucine/kg)	3 (12 g leucine + 6·8 g isoleucine 8·5 g valine/kg)
Present experiment:				
75	300	0/3*	0/8	0/4
120	(150 from	3/3	2/8	1/4
150	75th day)	3/3	6/8	3/4
Belavady et al. (1967):				
60	300	0/5	3/5	—
120	300	0/5	5/5	—

*The fourth pup would not eat the diet and was removed.

Our results gave no indication of leucine having an anti-nicotinic acid effect. Performance on diet 2 was at least equal to that on diets 1 and 3. We, as others (e.g. Axelrod, Morgan & Lepkovsky, 1945) used starch as being more palatable than the sucrose used by Belavady et al. (1967), though some puppies still failed to eat consistently. The Indian dogs were mongrels, caged individually on wire floors (to prevent coprophagy) and had food permanently in front of them. We cannot suggest how these differences could explain the discrepancy.

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The conversion of bound nicotinic acid to free nicotinamide on roasting sweet corn. By E. KODICEK* and D. R. ASHBY, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council, Cambridge CB4 1XJ* and M. MULLER and K. J. CARPENTER, *Department of Applied Biology, University of Cambridge, Cambridge CB2 3DX*

The traditional Central American practice of cooking maize with lime has the effect of hydrolysing the bound nicotinic acid to free nicotinic acid (cf. Kodicek, 1962). The Hopi Indians in Arizona have 'roasted' cobs of sweet corn of high moisture content in hot ashes and then dried them in the sun for storage until they are finally boiled (cf. Weatherwax, 1953). Sweet corn is at almost neutral pH, and it was therefore expected that these treatments would give little release of free nicotinic acid. The appearance of a high level of material corresponding to free nicotinamide on paper chromatograms of ethanol–water extracts was quite unexpected. The procedure (Kodicek & Wilson, 1959) depends on visual comparison and is only semi-quantitative, but the quantities (mg/kg dry matter) were considerable:

Material	Treatment	Total nicotinic acid	Free nicotinic acid	Free nicotin-amide
Hopi sweet corn	Roasted, dried, boiled and freeze-dried (1972)	23	6	17
	Freeze-dried (1973)	37	15	tr
	Roasted and freeze-dried (1973)	38	12	27
Frozen Canadian sweet corn	Freshly thawed	—	3	3·5
	Freeze-dried (with heat input to plates)	—	0	11
	Boiled 10 min and freeze-dried	55	2	55
	Roasted and freeze-dried	—	20	30
Ordinary maize meal	None	21	tr	0
	Boiled 3 h with sugars	—	4	0

tr, trace.

Similar results could be reproduced by roasting, or even by boiling, frozen sweet corn purchased in Cambridge. Sweet corn is a mutant of ordinary flint or dent corn which has reduced ability to accumulate starch and remains sugary (Smith, 1955) but cooking ordinary maize meal with added water and sugar has not resulted in the appearance of nicotinamide spots. Other samples of wheat, barley and oat grains did not show appreciable production of free nicotinic acid or amide. However, with immature barley grains significant production of nicotinamide was observed.

Bound nicotinic acid in cereals, in ester linkage with sugars (Mason & Kodicek, 1973), can be converted to nicotinamide by exposure to NH_3 vapour (Kodicek & Wilson, 1960). We do not yet know if this accounts for the changes in sweet corn, though NH_3 can be liberated from heated proteins (Bjarnason & Carpenter, 1970). However, it is of interest that another traditional way of using corn has been found

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to give a product of higher nicotinic acid value than ordinary maize meal cooked in European ways.

We thank Professor D. H. Calloway (University of California) and the Hopi women who collaborated with her in providing samples of their traditional foods.

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Relative affinities of isolated cell walls of rumen bacteria for calcium and magnesium ions. By T. J. FITT, K. HUTTON and W. R. OTTO, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

It has been suggested that the rumen microbial population may be responsible, at least in part, for the reduced availability of dietary magnesium observed in adult ruminants as compared with the pre-weaned ruminant or single-stomached livestock. The observation that cell walls prepared from rumen bacteria are capable of binding Mg offers support for this hypothesis (Fitt, Hutton, Thompson & Armstrong, 1972). The role of calcium as an essential nutrient is well known and there appears to be a relationship between the relative dietary concentrations of Ca, Mg and potassium and outbreaks of hypomagnesaemic tetany in cattle (see Grunes, Stout & Brownell, 1970). Metal ions such as Ca have a marked effect on the binding of Mg by bacterial cell walls. Observations with cell walls of Gram-negative organisms suggest that the molar proportions of Mg and Ca bound are not directly proportional to their respective molar concentrations in solution (Humphrey & Vincent, 1962), whereas results obtained with cell walls of Gram-positive organisms contradict these findings (Cutinelli & Galdiero, 1967; Archibald, Baddiley & Heptinstall, 1973).

In the present study, the relative affinities of isolated cell walls of rumen bacteria for Mg and Ca ions are reported. Cell walls were prepared from bacteria isolated from the rumen fluid of sheep and uptakes of Mg and Ca ions were measured at pH 6·5 by methods similar to those used previously (see Fitt *et al.* 1972). The results are shown in Fig. 1. It is clear that Mg uptake increased with increased Mg concentration, an observation which supports results already reported (Fitt *et al.* 1972). Furthermore, the uptake of Ca increased with increased Ca concentration. However, the
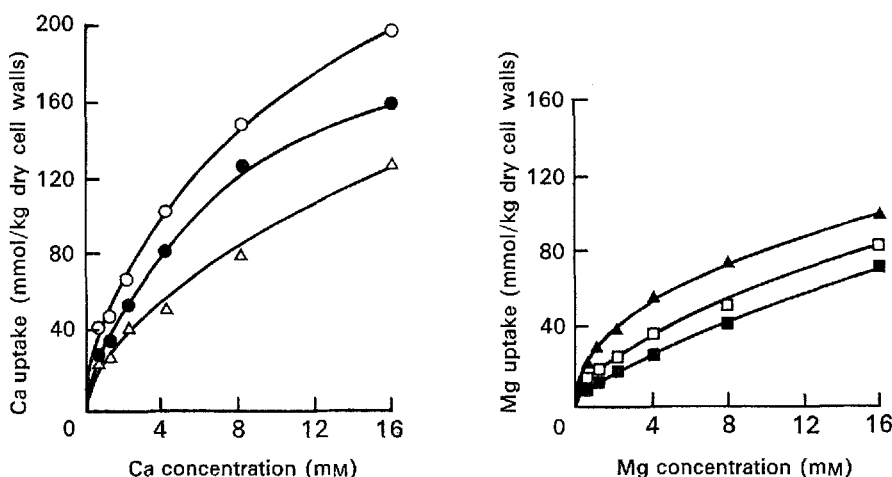
Fig. 1. Uptake (mmol/100 g dry cell walls) of calcium and magnesium by isolated cell walls of rumen bacteria. ○—○, Mg 0 mM; ●—●, Mg 4 mM; △—△, Mg 16 mM; ▲—▲, Ca 0 mM; □—□, Ca 4 mM; ■—■, Ca 16 mM.

relative quantities of Mg and Ca bound by the rumen bacterial cell walls were not in direct proportion to the molar concentrations of the respective ions in solution and there was a distinct preference for Ca ions.

The fact that uptake of Mg was substantially reduced in the presence of Ca emphasizes the potential nutritional significance of interactions between cations and the cell walls of micro-organisms in the digestive tract.

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Effect of potassium ions on the uptake of magnesium by isolated cell walls of rumen bacteria. By T. J. FITT and K. HUTTON, *Department of Agricultural Biochemistry, University of Newcastle upon Tyne*

The apparent availability of magnesium for sheep falls as the proportion of dietary potassium rises, inferring that the absorption of Mg is impeded (see Fontenot, Wise & Webb, 1972). Interactions between K, Mg and bacterial cells have been reported which may contribute towards an understanding of the effect of dietary K levels on Mg availability, but the results are difficult to interpret. No effects of K on Mg uptake by intact cells of *Escherichia coli* were observed by Silver (1969). However, K can displace wall-bound Mg and the concentration of K relative to Mg governs the extent of Mg binding (Strange & Shon, 1964).

The effects of varying levels of K on the uptake of Mg by isolated cell walls of rumen bacteria are reported in this study. The uptakes of Mg at different concentrations were similar to those reported previously for rumen bacterial cell walls (Fitt, Hutton, Thompson & Armstrong, 1972; Fitt, Hutton & Otto, 1974) and the effect of K was to reduce Mg uptake (see Fig. 1).
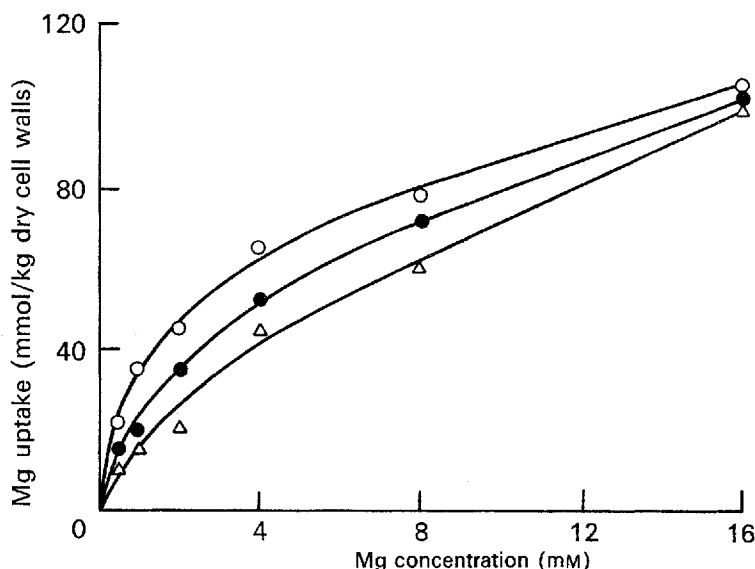

Fig. 1. Effect of potassium on the uptake of magnesium by isolated cell walls of rumen bacteria. ○—○, K 0 mM; ●—●, K 10 mM; △—△, K 50 mM.

The observation that K reduces the binding of Mg by isolated cell walls of rumen bacteria and that the effect is relatively small does not rule out the possibility that interactions between rumen micro-organisms, Mg and K are of relevance concerning Mg availability in ruminants. Intact rumen micro-organisms are our real concern and in this context it should be emphasized that an increase in dietary K level increases the intake of water (see MacFarlane & Howard, 1970) resulting in an increased dilution rate within the rumen. It is well established that the growth of micro-organisms per unit of ATP made available during oxidation of an energy source increases with dilution (see Hogan & Weston, 1970). The over-all result of such dilution in terms of mineral nutrition could well be an increased requirement for Mg by the rumen micro-organisms for their own metabolic processes, thus leading to a reduction in availability of dietary Mg to the host animal.

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The transfer and utilization of vitamin C in human tissues. By C. W. M. Wilson and M. Greene, *Department of Pharmacology, University of Dublin, Trinity College, Dublin 2, Ireland*

Ascorbic acid (vitamin C) is stored in the leucocytes and undergoes a circadian rhythm (Wilson, 1973). It is a physiologically active compound which is transferred rapidly backwards and forwards between the leucocytes and plasma, and between the plasma and tissues. Its utilization is affected by ageing (Wilson, 1974) and pathological conditions. The leucocyte–plasma transfer can be investigated after a loading dose of 2000 mg ascorbic acid by measuring plasma and leucocyte levels during a 4 h period, so as to assess physiological and pathological states. Normal female leucocytes absorb more ascorbic acid than those of males. Absorption continues until 4 h after the loading dose in females. It ceases in males after 2 h. During colds 2000 mg ascorbic acid causes a significant rise in the level in female leucocytes. It is greater than the post-cold rise. This dose does not raise male leucocyte levels. Plasma levels are raised more in the post-cold test in both sexes. Males and females do not differ in maximum leucocyte ascorbic acid concentrations after receiving adequate supplementation (Loh & Wilson, 1971). Females have a lower intake of ascorbic acid than males (Morgan, Gillum & Williams, 1955). Increased intake raises leucocyte concentrations; but the female sex differential is maintained The sex difference in response to dietary intake, or loading doses, of ascorbic acid is a metabolic characteristic. Normal guinea-pigs and human beings cannot synthesize ascorbic acid because they do not possess gulonolactone oxidase, which performs the synthesis of ascorbic acid from gulonolactone (Roy & Guha, 1958). Female guinea-pigs can survive for prolonged periods on scorbutogenic diets, and gulonolactone administration prolongs life during vitamin C deficiency (Odumosu & Wilson, 1973). D-Glucuronolactone is converted into ascorbic acid in man (Baker, Sauberlich, Wolfskill, Wallace & Dean, 1962). Thus humans may also synthesize the vitamin from suitable precursors (Atkins, Dean, Griffin & Watts, 1964). Women develop scurvy less than men during vitamin C deficiency (Wilson, 1974). Both species may therefore have the capacity to react to stress, or deficiency, by more economic utilization, and by synthesis, of ascorbic acid.

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Ascorbic acid metabolism in human cancer. By S. KAKAR and C. W. M. WILSON, *Department of Pharmacology, University of Dublin, Trinity College, Dublin 2, Ireland*

It has been demonstrated that tumour tissue in experimental animals has a high level of ascorbic acid during the period of rapid growth (Musulin, Silverblatt, King & Woodward, 1936), and blood ascorbic acid concentrations are low in patients with gastric carcinoma, though they are normal in patients with benign gastric tumours (Freeman & Hafkesbring, 1957). Leucocyte and plasma ascorbic acid values have been compared in normal children (4–14 years) and those with acute lymphatic leukemia (Table 1). Ascorbic acid intake was similar, and white cell counts

Table 1. *Plasma, leucocyte, skin and secondary cancer ascorbic acid values in children and geriatric subjects*

(Mean values and standard deviations where given)

	No. subjects	Leucocyte (μg/10^8 cells)	Plasma (μmol/l)
Children			
Control blood	10	56.4 ± 21.9	22.7 ± 11.4
Leukemic blood	10	35.9 ± 15.1	54.0 ± 14.2
P		<0.05	<0.001
Geriatric			
Control blood	7	26.0 ± 17.1	26.7 ± 11.4
Lung cancer blood	1	12.5	7.4
		Normal (μg/g)	Tumour (μg/g)
Skin biopsy	1	25	46

were normal, in the control and in the leukemic children. Duration of the leukemia was 1.5–3 years. Ascorbic acid concentrations were significantly lower in the leukemic children. In one male subject aged 60 suffering from pulmonary carcinoma for 5 months, leucocyte and plasma values were considerably lower than in normal geriatric subjects. The subject had numerous skin secondaries. Biopsy of a skin secondary showed that it contained almost twice as much ascorbic acid as unaffected adjacent skin. The content of adjacent unaffected skin was 31% of the normal skin concentration (81 μg/g; Barton, Laing & Barisoni, 1972). Plasma and tissue ascorbic acid concentrations are reduced in these two forms of cancer. Leucocyte levels are also diminished, suggesting that leucocyte ascorbic acid stores have been depleted. The ascorbic acid content of the secondary was higher than that of adjacent normal tissue. This suggests that growing tumour tissue in humans selectively concentrates ascorbic acid, thus accounting for the depleted leucocyte stores and reduced plasma levels of the vitamin in human cancer.

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The vitamin E content of selected infant milk formulas. By J. P. Le
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There is some evidence that premature infants are liable to develop a mild haemo-
lytic anaemia when fed on infant milk formulas which are either low in vitamin E
or have a low vitamin E:polyunsaturated fatty acid (PUFA) ratio (Lo, Frank &
Hitzig, 1973). We have analysed a number of infant milk formulas for vitamin E by
a modification of the method of Whittle & Pennock (1967).

Table 1 gives the mean vitamin E content and vitamin E:PUFA ratios of nine
infant milk formulas: eight available in the UK and one, Almiron B, having the
highest PUFA content of infant milks in the European Economic Community.
Vitamin E is expressed in terms of α-tocopherol equivalents; where vitamin E-active
compounds other than α-tocopherol were present, account was taken of their lower
biological potencies (e.g. relative to α-tocopherol $= 1 \cdot 00$, γ-tocopherol $= 0 \cdot 08$ and
α-tocotrienol $= 0 \cdot 21$).

Table 1. *Mean vitamin E content and vitamin E:polyunsaturated fatty acid (PUFA)*
ratios of infant milk formulas

Infant milk	Fat source*	Vitamin E† (mg/kg dry matter)	PUFA‡ (g/kg dry matter)	Vitamin E: PUFA ratio (mg/g)
Ostermilk One (Glaxo)	C	7·0	4·4	1·6
Ostermilk Two (Glaxo)	C	7·7	3·7	2·1
Golden Ostermilk (Glaxo)	C	5·0	4·8§	1·0
Babymilk One (Cow and Gate)	C	8·3	2·6	3·2
Babymilk Two (Cow and Gate)	C	8·0	4·8	1·7
Lactogen (Nestlé)	C	10·9	4·4§	2·5
SMA (John Wyeth)	F	80·7	71·7	1·1
V Formula (Cow and Gate)	F	102·1	32·5	3·1
Almiron B (Nutricia)	F	47·7	126·8	0·38
Human milk ‖	—	23·3	49·1	0·47

*C, cow's milk fat; F, filled milk.
†Vitamin E expressed in terms of α-tocopherol equivalents.
‡PUFA values taken as sum of 18:2 and 18:3 acids (from E. M. Widdowson & Y. Schutz, personal
   communication); for Cow and Gate V formula the manufacturer's value was used.
§Estimated from other infant milk formulas containing only cow's milk fat.
‖Values for human milk from McCance & Widdowson (1969).

The six formulas containing only cow's milk fat contain less vitamin E than
human milk. By this criterion they are therefore inadequate. However, with the
exception of Almiron B, all the milks are adequate, in comparison with human
milk, in terms of their vitamin E:PUFA ratios. The UK infant milks show a higher
vitamin E:PUFA ratio than is reported either in the USA (Davis, 1972) or in
Canada (Desai, O'Leary & Schwartz, 1972).

Standard vitamin E-active compounds were kindly provided by Roche Ltd.

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The toxicity of cow's milk to infant rabbits. By M. J. HENSCHEL and M. E. COATES, *National Institute for Research in Dairying, Shinfield, Reading RG2 9AT*

A high mortality rate has been experienced in our laboratory among hand-reared rabbit pups. It has been observed in animals given diets of evaporated milk, or reconstituted dried milk, or fresh cow's milk fortified with casein and vitamins and sterilized by an ultra-high-temperature process. There were two peaks of mortality, one between 7 and 10 d and one between 15 and 20 d of age. Measurements of enzyme activity showed that pancreatic protease output in artificially-reared, but not doe-suckled, rabbits fell markedly soon after birth (Henschel, 1973). By 10–12 d of age the protease level had regained that of the doe-suckled pups. The cause of this fall is not yet known, but survival during the first 10 d of life among hand-reared rabbits was enhanced by predigestion of the diet with a mixture of trypsin and α-chymotrypsin. However, the later peak of mortality still occurred. An apparently allergic and often fatal reaction to the diet developed, being more marked when the diet was predigested. This reaction has already been reported by Coates & O'Donoghue (1967).

To investigate the possible allergenicity of the milk diets, young adult guinea-pigs were sensitized with two intraperitoneal injections of 0·1 ml of digested or un-digested diet, and 3 weeks later were challenged intravenously with 0·01 to 0·02 ml of digested or undigested diet which had been centrifuged at high speed for two periods of 30 min. Reactions to the digested milk diet were at least as strong as to the undigested diet.

We believe that, since diets based on cow's milk are used so extensively as human milk replacers, this result warrants a further investigation into milk hypersensitivity in man, particularly in relation to its possible connection with sudden unexplained death in infancy.

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Effects of insulin and growth hormone on protein synthesis in newborn offspring of protein–energy malnourished rabbits. By K. A. ALLEN, K. A. MUNDAY and M. R. TURNER, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

New Zealand White rabbits were mated at 3·5 kg body-weight and fed throughout gestation on diets containing 200 g protein/kg (HP) or 100 g protein/kg (LP) derived from isolated soya-bean protein (Promine-D). The food intake during gestation was similar in both groups, so the LP animals were suffering from a protein insufficiency, but not an energy insufficiency.

Hemi-diaphragms from the newborn offspring were incubated for 1 h in the presence or absence of insulin or growth hormone. The accumulation into the total tissue water of [³H]arginine and of [¹⁴C]valine, and the incorporation of these amino acids into protein, were measured.

The effect of both growth hormone (GH) and insulin (I) on amino acid incorporation into protein was significantly reduced in the muscle from LP offspring (Table 1). Differences in amino acid incorporation were not due to changes in amino acid uptake into the tissue, which was at least as great in muscle from LP animals as in the control group.

Table 1. *Amino acid incorporation into protein and the percentage increment due to growth hormone (GH) and insulin (I) in muscle from rabbits given 200 g protein (HP) and 100 g protein (LP) per kg diet*

	Arginine incorporation			Valine incorporation		
	Basal rate (nmol/g fresh muscle per h)	Increment (%) due to presence of:		Basal rate (nmol/g fresh muscle per h)	Increment (%) due to presence of:	
Diet		GH	I		GH	I
HP	52·3	+60***	+40**	30·4	+56**	+35*
LP	37·8††	+29*†	+20**†	41·2††	+29**†	+12*†

Significance of differences: from basal rate: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; from HP: † $P<0.05$; ††, $P<0.01$.

The finding of a reduced protein-synthetic response to hormones in protein-deficient animals is of significance in the interpretation of alterations in hormone secretion in protein–energy malnourished (PEM) states. Thus an elevated plasma GH level such as commonly occurs in PEM does not necessarily mean that there is an increase in GH action at the tissue level. Indeed it could be that a reduced hormone–target-cell interaction leads to the elevation of the plasma GH levels. Similarly, a reduced tissue response to insulin will exacerbate the effects of the reduction in insulin secretion typical of most cases of PEM.

Effects of age and postnatal diet on the protein synthetic actions of insulin and growth hormone in rabbits. By K. A. ALLEN, K. A. MUNDAY and M. R. TURNER, *Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 3TU*

The incorporation of [³H]arginine and [¹⁴C]valine into protein, and the accumulation of these amino acids in the total tissue water has been measured in diaphragm muscle from rabbits at birth, at weaning and after being fed for 18 weeks from weaning on a stock diet (RAG, Christopher Hill Ltd, Poole) or on diets containing (/kg) 200 g (HP) or 100 g (LP) protein derived from soya bean (Promine-D). The food intake of HP and LP groups was similar at all ages.

The basal rate of incorporation of amino acid into protein was high in the new-born, but declined with age. The percentage increase in amino acid incorporation due to growth hormone (GH) was similar at all ages. In the presence of insulin, on the other hand, there was an increase with age in amino acid incorporation, an effect which could be explained at least in part by changes in the nature of the tissue response to insulin in different age groups. In the newborn, insulin caused a reduction in the accumulation of free amino acids in the tissues, it had no effect on the weanling, but stimulated amino acid accumulation in the adult. GH had an effect on amino acid accumulation only in the newborn.

In tissue from adult LP rabbits, the effects of insulin on amino acid accumulation was lost. The protein synthetic response to both insulin and GH tended to be reduced ($0 \cdot 1 > P > 0 \cdot 05$), but the loss of tissue responsiveness to hormones in post-weaning protein deficiency was less marked than that observed in congenital protein deficiency (Allen, Munday & Turner, 1974) and to GH in more severe, short-term protein restriction in the adult (Turner, Reeds & Munday, 1974).

The implications of these observations will be discussed.

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Glucose uptake by the perfused hind–limb of rats: the effect on insulin sensitivity of diets of different protein–energy value. By L. ASATO, C. R. C. HEARD and R. D. SKETCHER, *Clinical Nutrition and Metabolism Unit, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

The perfused hind-limb (Jefferson, Koehler & Morgan, 1972) permits the study of whole muscle metabolism under conditions of controlled nutrient and hormonal supply. In the present work the perfusion medium (approximately 60 ml) contained washed human erythrocytes, bovine serum albumin, amino acids, glucose ($5 \cdot 55$ mmol/l), and bovine insulin (1 mU/ml) in Krebs-Henseleit bicarbonate buffer. After recycling of the oxygenated medium had commenced, portions were taken every 5 min for glucose estimation. Extra glucose ($0 \cdot 2$ ml of $1 \cdot 11$ mol/l solution) was added after 60 min. Glucose disappearance from the medium followed first-order kinetics, and rate constants (k) were calculated where $k =$ percentage glucose removed per minute (% per min). Because of difference in perfusion volume (V) and muscle (hind-limb) mass (M), all k values were corrected to unit volume and mass, i.e. k (corrected) $= k$ (observed) $\times$ V/M.

The animals used in the experiments were black-and-white hooded Lister rats, (about 80 g initial weight) which had been fed for 4 or more weeks on one of the following: (a) diet with ratio of protein energy : total energy (NDp:E) $= 0 \cdot 10$, *ad lib.* (HP), (b) diet with NDp:E $= 0 \cdot 04$, *ad lib.* (LP), (c) HP diet in amounts pair-fed to

intake of LP diet (HP-PF). The biochemical effects of these dietary regimens have been reported (Heard, Frangi & Wright, 1973). An additional group of rats was fed on the HP diet until their mean body-weight was equal to the mean weight of LP rats at the time of experiment (HP-weight control). These animals were therefore younger than those of the other three groups.

In each experiment three k values were calculated: (a) 'k-lag' covered an initial period (about 23 min) when glucose consumption rate was low, (b) 'k-I', the following 30–40 min, when the effect of insulin was apparent, (c) 'k-G', the final 30 min after adding glucose.

In all groups k-I was equal to k-G, giving two values for insulin sensitivity. In all groups also, the ratio 0.5 (k-I + k-G): k-lag was the same (2.34 ± 0.13), a correlation between insulin-stimulated glucose uptake and basal glucose uptake similar to that found in the whole animal (Heard & Henry, 1969).

Mean values for k-I for the four groups of rats (five or six rats/group) were (% per min): HP, 1.50; LP, 3.21; HP-PF, 3.11; and HP-weight control, 6.39. All differences were significant ($P < 0.01$) except between LP and HP-PF. These mean values for k-I correlated significantly ($r = 0.95$; $P < 0.01$) with mean k values for intravenous glucose tolerance in living animals, and demonstrate changes in insulin sensitivity related to both age and diet. The fate of the glucose, and correlations of glucose uptake with other metabolic measurements have still to be investigated.

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The effect on muscle protein metabolism of refeeding protein–depleted rats. By D. J. MILLWARD, D. O. NNANYELUGO and P. J. GARLICK, *Clinical Nutrition and Metabolism Unit, Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1E 7HT*

In skeletal muscle of malnourished rats protein synthesis is severely depressed because of a loss of synthetic capacity (RNA: protein ratio) and a reduction in efficiency (synthesis rate: RNA). It follows therefore that rehabilitation from a poor diet requires a restoration to normal levels of these two measures of protein synthesis. We have previously reported a rapid restoration of the efficiency of muscle protein synthesis on refeeding with a good diet (Millward, Garlick, James, Nnanyelugo & Ryatt, 1973). We have now extended these studies with measurements of capacity and efficiency of protein synthesis in the gastrocnemius and quadriceps muscle during a 2-week period of rehabilitation. We have also measured the growth rate of muscle protein so that the rate of breakdown can be calculated. The results, which are summarized in Table 1, show that not only was the efficiency of protein

synthesis restored to normal levels after 1 d of refeeding, but the efficiency increased to higher than control values during the rehabilitation. As a result of the restored capacity and elevated efficiency of protein synthesis, the rate of protein synthesis was elevated above control values after 8 d of refeeding. At this time the breakdown rate of muscle protein was also increased. This increase in breakdown rate during rehabilitation will be discussed together with divergent results from other workers.

Table 1. *Protein metabolism in skeletal muscle of rats*

(Mean values with their standard errors where given)

Diet	Day	Muscle protein (mg)	Ratio RNA: protein ($\times 10^8$)	Growth rate of muscle protein* (/d)	Rate of protein synthesis* (/d)	Efficiency of synthesis (g protein/ g RNA per d)	Protein break-down rate*† (/d)
Protein-free	30	51 ± 4	$3 \cdot 3 \pm 0 \cdot 2$	$-0 \cdot 030$	$0 \cdot 027 \pm 0 \cdot 009$	$8 \cdot 0 \pm 2 \cdot 6$	$0 \cdot 057$
High-protein: refed	1	52 ± 4	$3 \cdot 9 \pm 0 \cdot 3$	$0 \cdot 043$	$0 \cdot 059 \pm 0 \cdot 001$	$15 \cdot 4 \pm 2 \cdot 9$	$0 \cdot 016$
	3	59 ± 4	$6 \cdot 3 \pm 0 \cdot 4$	$0 \cdot 049$	$0 \cdot 104 \pm 0 \cdot 020$	$16 \cdot 6 \pm 3 \cdot 0$	$0 \cdot 055$
	8	74 ± 6	$10 \cdot 4 \pm 1 \cdot 3$	$0 \cdot 068$	$0 \cdot 198 \pm 0 \cdot 028$	$19 \cdot 2 \pm 3 \cdot 2$	$0 \cdot 130$
	14	115 ± 22	$8 \cdot 3 \pm 0 \cdot 3$	$0 \cdot 061$	$0 \cdot 171 \pm 0 \cdot 017$	$20 \cdot 3 \pm 1 \cdot 7$	$0 \cdot 110$
Weight controls		81 ± 4	$10 \cdot 3 \pm 0 \cdot 5$	$0 \cdot 050$	$0 \cdot 134 \pm 0 \cdot 026$	$13 \cdot 8 \pm 3 \cdot 2$	$0 \cdot 084$
Age controls		207 ± 24	$5 \cdot 1 \pm 0 \cdot 3$	$0 \cdot 007$	$0 \cdot 078 \pm 0 \cdot 012$	$15 \cdot 4 \pm 2 \cdot 5$	$0 \cdot 071$

*Fractional rate constant.
†Calculated as: synthesis rate – growth rate.

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