Five groups of four litter-sister gilts were reared under standard conditions and were mated when they weighed approximately 130 kg. During the first 50 days after mating they received a basal ration of 1.35 kg/day of a diet with 14.1% crude protein and 4100 cal/g dry matter. From the 50th to the 110th day of pregnancy animals were given (1) the basal ration of 1.35 kg; (2) the basal ration plus 0.95 kg maize starch daily; (3) the basal ration plus 0.15 kg casein daily or (4) the basal ration plus 0.95 kg starch and 0.15 kg casein. Nitrogen retention was increased by 25% with the addition of starch, by 69% with the addition of casein and by approximately 92% when starch and casein were both added. No relationship was found between N retention during pregnancy and the intrauterine deposition of N.

## Circadian rhythm of rumination. By J. G. GORDON, Rowett Research Institute, Bucksburn, Aberdeen

The effect of light in determining the pattern of incidence of rumination was discussed.

The One Hundred and Eightieth Meeting of The Nutrition Society was held in the Sir John Atkins Laboratories, Queen Elizabeth College, Campden Hill, London, W 8, on Saturday, 21 May 1966, at 10 am, when the following papers were read:

## Comparison of two simple measures of activity in old women. By Molly M. Disselduff and Edna Murphy, Ministry of Health, London, SE 1

Comparisons were made, on ten women aged 75-95, all senile to the point where institutionalization was imminent, between the reading of a pedometer attached to their day clothes for 24 h, and their activity as indicated by the relationship between their basal metabolic rates and their total caloric intake measured by 7-day dietary records prepared under close and repeated supervision. No attempt was made to measure the distances walked; the pedometer record simply related to the number of movements made in the day of sufficient magnitude to register on the pedometer.

The relationship found was sufficiently close to justify the conclusion that the two methods of assessment were in effect measuring the same thing, albeit in different ways, viz, the amount of movement made. Indeed it was unexpectedly close, partly it is thought because, compared with younger subjects, these old women lived very uniform lives with little variation from week to week either in the food they ate or the activity which they took. They may thus have avoided the short-term discrepancies between caloric expenditure and consumption often reported in

younger subjects. The other possible reason for the closeness of the relationship found was that in contrast perhaps to some younger subjects, most of them were performing tasks for which they needed to husband their strength and in which, through repetition over the weeks and months, they had learnt to exercise the utmost economy.

Three women registered zero upon the pedometer. Though all indulged in a certain amount of activity, their movements were so gradual and restricted that the pedometer failed to record.

### A study of the role of iodine deficiency in simple goitre in the Glasgow area.

By W. R. Greig, J. A. Boyle, Anne Duncan, Janette Nicol, Mary Gray, W. W. Buchanan and E. M. McGirr (introduced by H. N. Munro), University Department of Medicine and the Department of Biochemistry, Royal Infirmary, Glasgow and the Centre for Rheumatic Diseases, Glasgow

While dietary iodine deficiency is responsible for goitre formation in some endemic goitre areas, the role of iodine deficiency in the causation of simple goitre in Western Europe is much less certain. Evidence has been offered from the study of hospital patients to support the concept that iodine deficiency, defined by a low level of plasma inorganic iodine (PII), does indeed co-exist with simple goitre (Alexander, Koutras, Crooks, Buchanan, MacDonald, Richmond & Wayne, 1962). A twin study performed recently in Glasgow in mainly young healthy individuals afforded an opportunity of correlating individual PII levels with the presence or absence of goitre in a non-hospitalized population. PII was measured indirectly by a technique based on a comparison of serum creatinine and urine iodine and creatinine concentrations. This method had previously been shown both by ourselves (Boyle, Sloss, MacDonald & Gray, 1965; Harden, Mason & Buchanan, 1965) and by others (Vought, London, Lutwak & Dublin, 1963) to give an acceptable correlation with a more standard isotope dilution technique of PII measurement.

The subjects studied were derived from the general population by an ascertainment campaign conducted in local hospitals, in the schools, on radio and on television. Three hundred and forty-four subjects were examined for the presence or absence of goitre, defined in this study as a palpable and visible thyroid enlargement which could be seen and felt by two independent observers; in 231 individuals the PII was estimated. The overall goitre rate for 139 males assessed for goitre was 9.4% and for 205 females it was 43.4%

The mean PII and standard deviation was  $0.25 \pm 0.19$  mg/100 ml in the eighty-nine non-goitrous females in whom it was measured compared with a value of  $0.21 \pm 0.15$   $\mu$ g/100 ml in fifty-eight goitrous women. This difference is not statistically significant (0.1 > P > 0.05). The PII values for seventy-six non-goitrous and eight goitrous men were  $0.23 \pm 0.17$  and  $0.22 \pm 0.15$   $\mu$ g/100 ml respectively. It would appear therefore that simple goitre in healthy young subjects may be due to factors other than deprivation of dietary iodine.

#### REFERENCES

Alexander, W. D., Koutras, D. A., Crooks, J., Buchanan, W. W., MacDonald, E. M., Richmond, M. H. & Wayne, E. J. (1962). *Q. Ji Med.* 31, 281.

Boyle, J. A., Sloss, A., MacDonald, E. M. & Gray, M. (1965). *J. clin. Endocr. Metab.* 25, 1035.

Harden, R. McG., Mason, D. & Buchanan, W. W. (1965). *J. Lab. clin. Med.* 65, 500.

Vought, R. L., London, W. T., Lutwak, L. & Dublin, T. D. (1963). *J. clin. Endocr. Metab.* 23, 1218.

# Influence of EDTA on the digestibility of calcium in rats. By G. VARELA and A. MURILLO, Laboratory of Animal Physiology, University of Granada, Spain

We have studied the influence of different levels of EDTA on calcium rendered indigestible by the presence of oxalate. The formation of chelates of EDTA with this insoluble calcium might increase the digestibility of this cation.

Eight experiments were carried out, using in each five male and five female rats, given a synthetic diet with a 12% protein content (bovine liver, boiled and washed in order to eliminate the calcium). The quantities of calcium carbonate, sodium oxalate and disodium EDTA, as shown in Table 1, were added to the basic diet, which did not contain added calcium.

Table 1. Effect of EDTA on the digestibility for rats of calcium in the presence of sodium oxalate (mean values with their standard errors for groups of ten rats)

		Addition (	% of diet)	Digestibility of Ca (%)		
Lot no.	Expt no.	Calcium carbonate	Sodium oxalate	Apparent	True	
I	1	_			_	
	2	I	<del></del>	46·50±2·12	49.43 ± 1.8	
2	1	_		-		
	2	I	0.75	15·30±1·7	17.98 ± 1.75	
	3	1	1.34	<0	0	
3	1			_		
	2	I	0.75	29·01±1·71	33·39±1·89	
	3	ı	1.34	17·37±1·54	22·77±0·73	

The coefficients of digestibility were determined by the standard technique, using individual metabolic cages for rats according to the model of K. Schiller modified for adult rats.

The digestibility of calcium (49.43%) was reduced to 17.98% due to the presence of oxalate (0.75%) in the diet; when the quantity of oxalate was greater (lot 2, Expt 3) digestibility was nil, which was to be expected since the quantity of oxalate present was calculated to precipitate all the calcium of the diet.

EDTA increased, to a significant degree, the true and apparent digestibility of the calcium in the presence of oxalate, as the complexone increases digestibility from 17.98 to 33.39 at level 1 of oxalate; and from 0 to 22.77 at level 2.

We can therefore conclude that EDTA is capable of mobilizing calcium and making it digestible when it is insoluble in the presence of oxalate.

Extent of human vitamin A deficiency. By D. S. McLaren, W. W. C. Read and Marie Tchalian, Nutrition Research Laboratory, American University of Beirut, Beirut, Lebanon

Estimates of the extent of nutritional deficiency are needed if prevention is to be planned. The problem of vitamin A deficiency is virtually confined to the preschool child and the severe ocular manifestations are the end result of a long process of depletion (Oomen, McLaren & Escapini, 1964).

In Jordan (population 1.7 million) two independent sets of data obtained from (1) a country-wide notification system of xerophthalmia and (2) hospital admissions; taken with recent census data on children <6 years gave similar figures: 238 and 132 respectively xerophthalmia cases annually. Extrapolation to the Middle East (population 125 million) and to the world's underprivileged (≤1000 million) using the smaller figure for Jordan gives about 10000 and 80000 respectively. A previous estimate with Indonesian data gave at least 20000 for the world (McLaren, 1963).

For evaluating vitamin A status the usual clinical, biochemical and dietary survey data have proved of little value. By estimating subnormal liver storage an attempt has been made to assess the magnitude of the part of the 'iceberg' of poor vitamin A status beneath the overt clinical disease. A micromethod (Neeld & Pearson, 1963) was applied to Menghini needle biopsy material and serum obtained within 4 days of admission from twenty-eight Jordanian children (1–3 years old) with protein-calorie malnutrition without eye lesions, and from thirty-six children of the same age recovered from non-nutritional conditions.

In both groups there was no correlation between liver and serum levels. In the control group there was a wide range of liver levels (mean  $71\cdot2\pm69\cdot7~\mu g/g$ ). Levels were significantly lower in girls ( $P\leqslant0.05$ ) perhaps due to cultural factors. Significantly lower levels were found in the malnourished (mean  $17\cdot6\pm24\cdot3~\mu g/g$ ;  $P\leqslant0.01$ ). Taking the range ( $15-80~\mu g/g$ ) found in children dying accidentally (Ellison & Moore, 1937) 8/36 of the control and 20/28 of the malnourished fell entirely below this range, suggesting that many of both groups are 'at risk'.

### REFERENCES

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Ellison, J. B. & Moore, T. (1937). Biochem. J. 31, 165.
McLaren, D. S. (1963). Malnutrition and the Eye. New York: Academic Press Inc.
Neeld, J. B. & Pearson, W. N. (1963). J. Nutr. 79, 454.
Oomen, H. A. P. C., McLaren, D. S. & Escapini, H. (1964). Trop. geogr. Med. 16, 271.
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# Ruminant faecal fat. By R. Andrews and D. Lewis, University of Nottingham School of Agriculture

In the determination of fat digestibility it is usual to apply a correction to apparent digestibility coefficients based on the assumption that the faecal fat of animals receiving diets containing fat can be divided into two fractions. One is considered to be of immediate dietary origin and represents that part of the ingested fat which has not been absorbed from the alimentary tract. The other is not regarded as being an immediate consequence of the diet and may result from intestinal secretions,

bacterial synthesis, bacterial residues or sloughing off of the mucosal cells. It is likely that the contribution of this second fraction to total faecal lipids is of considerable significance in the determination of lipid digestibility coefficients in ruminants.

In an investigation of this aspect, lipid outputs by sheep have been assessed on a low-fat basal ration consisting of hay, barley and extracted soya-bean meal, and on an equivalent ration supplemented with 7.0% of beef tallow. An examination of the faecal lipids on each ration has been made by techniques of thin-layer and gas—liquid chromatography.

When sheep of 40 kg live weight received 1200 g/day of the low-fat ration the total faecal output was equivalent to 7.5 g/day. The distribution within the faecal fat of free fatty acids, mono-, di- and tri-glycerides, sterols, sterol esters and phospholipids has been examined. The proportions of these components remained relatively constant over a period of 75 days. Analysis by gas-liquid chromatography showed that stearic and palmitic acids were predominant. Isomers of pentadecanoic and heptadecanoic acids were also important but little oleic acid was detected.

Faecal lipid output rose rapidly to a maximum of 44 g/day 2 days after the introduction of the fat-supplemented ration and decreased to a minimum of 9 g/day after 20 days. The increased output of faecal lipid was due almost entirely to an increase in free fatty acids in the faeces although a small increase in triglyceride output was apparent over the first few days. Total lipid output showed considerable variation over a period of 75 days again mainly due to variation in the free fatty acid content of the faeces. Chromatographic analysis demonstrated a marked increase in faecal stearic and palmitic acids but little increase in oleic acid.

# The passage of vitamin C across biological membranes. By R. E. Hughes and S. C. Maton, Welsh College of Advanced Technology, Cardiff

Because of their activity in retarding the breakdown of vitamin C in whole blood the erythrocytes make an important contribution to the vitamin C economy of the body. Dehydroascorbic acid (DHA), the first breakdown product of ascorbic acid (AA), is reduced back to AA by a system located in the erythrocyte (Hughes, 1964; Hughes & Kilpatrick, 1964). The experiments reported here were designed to cast further light on the factors affecting the passage of vitamin C across the erythrocyte membrane.

Previous workers have established that vitamin C enters the human erythrocyte in the oxidized form as DHA (see e.g. Räihä, 1958). Using washed erythrocytes resuspended at  $37^{\circ}$  in isotonic phosphate buffer containing either AA or DHA, we have shown that this is also true in the case of the sheep, ox, pig, rat, kitten and guinea-pig. The uptake was pH-dependent but was unaffected by ouabain, sodium azide, phloridzin and p-chloromercuribenzoate when present at a concentration of  $M^{-3}$ .

Dehydroisoascorbic acid (dehydro-D-araboascorbic acid) did not enter the erythrocyte under conditions that permitted a rapid uptake of DHA; furthermore,

there was no reduction of DHA uptake in the presence of dehydroisoascorbic acid. The rank order of uptake for the species examined was guinea-pig>rat>kitten>ox > sheep; it would appear that these different rates of uptake are at least in part attributable to the different activities of the erythrocyte 'DHA-reductase' system in the different species (Hughes, 1964).

The rate of release of ascorbic acid from the erythrocyte was found to be much less rapid than the rate of uptake of DHA under the same conditions.

Similar experiments designed to examine the uptake of AA by the isolated eye lens produced a quite different picture. In the case of the lens the vitamin entered in the reduced form, there being no appreciable uptake of DHA.

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### REFERENCES

Hughes, R. E. (1964). *Nature, Lond.* 203, 1068. Hughes, R. E. & Kilpatrick, G. S. (1964). *Clinica chim. Acta* 9, 241. Räihä, N. (1958). *Acta physiol. scand.* 45, Suppl. 155.

## The effect of infusing bile salts into the rumen of sheep. By J. L. CLAPPERTON,

J. W. CZERKAWSKI and A. K. MARTIN, Hannah Dairy Research Institute, Ayr

When sodium salts of long-chain fatty acids are infused into the rumen of sheep the methane production of the rumen falls. When other surface-active agents such as sulphated fatty alcohols are infused methane production falls and faecal energy increases (Czerkawski, Blaxter & Wainman, 1966). It was interesting, therefore, to see whether bile salts, naturally occurring surface-acting agents, would have any effect if infused into the rumen.

Sodium tauroglycocholate (BDH Ltd, prepared from ox bile) was infused into the rumen of sheep at the rate of 2–8 g/day. The sheep were offered either dried grass or a concentrate ration at about the maintenance level. In some experiments, the animals were confined in a respiration chamber, in others they were in metabolism cages. In all the experiments faeces and urine were collected. Rumen samples were obtained from the sheep via a rumen cannula and were analysed for total volatile fatty acids by steam distillation and for the individual acids by the method of James & Martin (1952).

The results showed that the infusion of bile salts had no effect on the heat production of the sheep and only a small effect on the methane production. The latter rose initially and then fell continuously as the infusion was continued. The digestibility of the dry matter, energy and nitrogen of the food was not affected although there was a lot of variation in the values for nitrogen digestibility. In most sheep, bile-salt infusion did not affect the urinary nitrogen excretion but in one animal the value rose from 10 to 24 g/day as the rate of infusion was increased. There was no effect on the concentration of the volatile fatty acids in the rumen liquor but, on the dried grass ration, the proportion of formic acid in the liquor rose from an initial value of 3.5 to 9.7% at the start of the infusion period and then fell as the rate

of infusion was increased to 3.5%. The proportion of acetic acid in the rumen liquor fell as the formic acid rose but the proportions of propionic and butyric acids were not affected. In the sheep given the concentrate ration there were only traces of formic acid in the rumen liquor.

In every experiment, as soon as the amount of bile salt infused reached 8 g/day, the sheep refused food and the effect persisted for periods of 10 days or more. Harrison (1962) has shown that when bile salts are infused into the jejunum, the rate of secretion of bile from the gall-bladder is increased and he ascribed the effect to the existence of an entero-hepatic circulation of bile salts. If the bile salts infused into the rumen in these experiments passed unchanged into the lower parts of the intestine, they might have exerted a similar effect so causing the animal to refuse to eat.

### REFERENCES

Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W. (1966). Br. J. Nutr. 20, 349. James, A. T. & Martin, A. J. P. (1952). Biochem. J. 50, 679. Harrison, F. A. (1962). J. Physiol., Lond. 162, 212.

Studies on protein deficiency and calorie deficiency in rats. 1. Plasma amino acid ratios and urinary hydroxyproline excretions. By Elsie M. Widdowson and R. G. Whitehead, Medical Research Council Department of Experimental Medicine, University of Cambridge

Young rats 4 weeks old were made protein-deficient by feeding them unlimited amounts of diet containing 5% protein or calorie-deficient by feeding them restricted amounts of a diet containing 20% protein so that their mean weight was the same as that of the animals having the low-protein diet. After 2 weeks the rats having the low-protein diet showed an abnormality in the ratio of inessential to essential amino acids in the plasma similar to that found in protein-deficient children (Whitehead, 1964), and the abnormality persisted so long as the animals remained on the protein-deficient diet. The calorie-deficient rats did not exhibit this abnormality to anything like the same degree.

The urinary excretion of hydroxyproline peptides was low in all the deficient animals, whatever the limiting factor in their diet. Whitehead (1965) described a test which can be applied to random samples of urine from children and involves the ratio of the concentration of hydroxyproline to creatinine. This ratio was also low in both groups of malnourished rats.

As with children with kwashiorkor, treatment of protein-deficient rats with plentiful amounts of a good diet quickly restored their fasting plasma amino acid ratio to normal. In both protein- and calorie-deficient animals there was a similar improvement in hydroxyproline excretion and hydroxyproline creatinine ratio, which was associated with a rapid rate of growth.

### REFERENCES

Whitehead, R. G. (1964). Lancet i, 250. Whitehead, R. G. (1965). Lancet ii, 567.

Studies on protein deficiency and calorie deficiency in rats. 2. Haematocrit, and haemoglobin and plasma protein concentrations. By Elsie M. Widdowson and J. W. T. Dickerson, Medical Research Council Department of Experimental Medicine, University of Cambridge

The disease known as kwashiorkor in children is characterized by a low level of plasma proteins, due principally to a fall in the concentration of albumin. The globulins are reduced much less, and they thus form a greater than normal percentage of the total plasma proteins. The children are often also anaemic. Frequently, however, there is a concurrent infection, which may affect the findings.

The protein- and calorie-deficient rats described in the previous paper (Widdowson & Whitehead, 1966) were used in the present investigation. Animals were bled from the abdominal aorta at weekly intervals during the 4 weeks of deprivation, and the 5 weeks of rehabilitation; and rats were also studied 3 days after rehabilitation began. Haemoglobin and haematocrit were measured, and the protein fractions in the plasma were separated electrophoretically.

During the 9 weeks of the experiment haemoglobin concentration and haematocrit increased steadily in the well-nourished control animals. During the 4 weeks of deprivation the levels remained unchanged in the calorie-deficient rats, but they fell precipitously during the first 3 days of rehabilitation, probably due to a rapid expansion of plasma volume, and this was followed by a steady rise after the end of the 1st week.

The haemoglobin and haematocrit levels fell in the animals having a proteindeficient diet and rose again during rehabilitation.

The concentration of total protein and of albumin rose in the well-nourished control animals during the period of the experiment. The values remained constant in those that were calorie-deficient and fell in the protein-deficient ones. The changes in globulin fractions were small in all groups. In the protein-deficient rats the response to rehabilitation was immediate and in 3 days the concentrations of total protein and of albumin rose by 1 g/100 ml. Rehabilitation of the calorie-deficient animals produced very little change in the plasma proteins.

### REFERENCE

Widdowson, E. M. & Whitehead, R. G. (1966). Proc. Nutr. Soc. 25, xxxi.

The absorption of vitamin A after a meal of liver. By I. M. Sharman and T. Moore\*, Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council, and Z. A. Leitner, 52 Welbeck Street, London W 1

Liver is outstanding among common foods as a rich source of vitamin A; sheep liver, for example, contains about 450 i.u./g (Moore & Payne, 1942). This means that 250 g, a not unusually large portion to be served at a meal, would be equivalent to a dose of 110000 i.u. vitamin A. Experience has shown that when doses of this magnitude are ingested by human subjects in the form of synthetic vitamin A, or

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of concentrates of the vitamin, or other therapeutic preparations, the absorption of the vitamin is indicated by a large increase in the level of vitamin A in the blood plasma. This increase usually reaches its peak after 4 or 5 h and the blood vitamin A usually returns to its resting level after 24-48 h. If vitamin A is equally well absorbed from liver as from therapeutic preparations, therefore, meals of liver should also result in large increases in the vitamin. This possibility interested us in view of the occasional observation of unexpectedly high vitamin A levels in the course of dietary surveys, and the possibility that these high values might sometimes be caused by a meal of liver (Leitner, Moore & Sharman, 1960). In view of the lack of evidence about the efficiency of absorption of vitamin A from liver it seemed desirable to carry out feeding tests with liver. Blood specimens were collected from three normal subjects before and at various times after eating 6-8 oz liver. These were analysed for vitamin A by the SbCl<sub>3</sub> method. Vitamin A was also determined in the liver that had been consumed and the total intake of the vitamin calculated. The results shown in the table indicate that substantial rises in the blood levels of vitamin A occurred following the meals of liver and then later subsided.

	Meal of sheep's liver		Blood vitamin A (i.u./100 ml) h after meal				
Subject	Wt (g)	Vitamin A (i.u.)	<u> </u>	4	6	24-28	72-75
R	170	130 000	270	510	430		
R*	170	150 000	250	840		270	150
$\mathbf{w}$	170	170 000	130	410	300		
G	230	360 000	200	820		430	220

<sup>\*</sup>Same subject as one above, re-examined 1 month later.

### REFERENCES

Leitner, Z. A., Moore, T. & Sharman, I. M. (1960). Br. J. Nutr. 14, 157. Moore, T. & Payne, J. E. (1942). Biochem. J. 36, 34.

# Investigations of the passage of α-linked glucose polymers into the duodenum of the sheep. By J. C. MacRae and D. G. Armstrong, University of Newcastle upon Tyne

It is generally believed that as a consequence of microbial fermentation in the rumen, enzyme digestion of carbohydrate material in the small intestine of the ruminant animal is of little importance. Heald (1951) and Weller & Gray (1954) feeding roughage diets, in the latter instance when supplemented with 148 g starch, to sheep reported respectively the passage of 5 g and 7.8 g of glucose per 24 h through the abomasum. Porter & Singleton (1965) using hay-fed goats reported a figure of approximately 3 g glucose per 24 h passing through the duodenum. There is no information as to the possible significance of carbohydrate digestion in the small intestine of the ruminant animal fed high-concentrate rations. Some results of a study designed to obtain quantitative data concerning the  $\alpha$ -linked glucose carbohydrates which pass into the lower digestive tract of the sheep fed an all-roughage ration or one high in grain are presented.

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Sheep fitted with duodenal re-entrant cannulas of the type described by Ash (1962) have been used. During each 24 h collection period the total duodenal contents were collected each 90 min, and representative samples of these collections analysed separately for  $\alpha$ -linked glucose polymers, and total reducing sugars. The method developed for measurement of  $\alpha$ -linked glucose polymers involves enzyme hydrolysis of the material with an amyloglucosidase prepared from Aspergillus niger fermentation broths (sold commercially as Agidex; Glaxo Research Ltd) followed by estimation of the glucose by the oxidase method of Marks (1959). Total reducing sugars were estimated after conventional acid hydrolysis (1%  $\rm H_2SO_4$ ) by the Somogyi (1945) method.

The data obtained from each of two sheep fed (a) a diet of 900 g hay (777 g dry matter) per 24 h in two feeds, and (b) a diet of 200 g hay (179 g dry matter) and 700 g rolled barley (570 g dry matter) per 24 h in two feeds are presented in the table.

			Analysis of duodenal contents (g/24 h)			
Diet Hay	Sheep W S	Dry-matter digestibility (%) 62.9 62.8	Dry matter 405·5 445·9	Glucose from $\alpha$ -linked polymers 1.41 2.32	Total reducing sugars 42.47 48.06	
Hay + barley	W S	81·4 82·3	271·7 267·6	16·76 21·95	41·98 48·48	

The data show that on both rations considerable amounts of carbohydrate polymers are present which on mild acid hydrolysis yield non-glucose reducing sugars. Examination of these by paper chromatography has shown that the major constituent is xylose with smaller amounts of arabinose and traces of galactose and ribose. On rations high in cereal the amount of  $\alpha$ -linked glucose polymer is appreciable and work is at present in progress on its subsequent fate. In view of the importance of glucose in ruminant metabolism (Armstrong, 1965) such material may make a useful contribution to the supply of this essential metabolite.

#### REFERENCES

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Armstrong, D. G. (1965). In Physiology of Digestion in the Ruminant, p. 272. [R. W. Dougherty, editor.] Washington, DC: Butterworths.
Ash, R. W. (1962). J. Anim. Prod. 4, 309.
Heald, P. J. (1951). Br. J. Nutr. 5, 84.
Marks, V. (1959). Clinica chim. Acta 4, 395.
Porter, P. & Singleton, A. G. (1965). Biochem. J. 96, 59P.
Somogyi, M. (1945). J. biol. Chem. 160, 61.
Weller, R. A. & Gray, F. V. (1954). J. exp. Biol. 31, 40.
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# The application of FAO protein allowances to British data. By J. P. Greaves and Jane Tan, Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, SW 1

In both FAO's reports protein requirements were expressed initially in terms of reference protein, and procedures recommended for making adjustment for the

value of dietary protein by calculating chemical scores. In the first report (FAO, 1957) scoring was based on the extent to which the limiting essential amino acid (EAA) in the dietary protein compared with its content in the provisional reference pattern; in the second (FAO, 1965), on the extent to which the limiting EAA, as a percentage of the total EAA (an A:E ratio) did so, the reference pattern adopted being changed to that of whole egg. Both reference patterns were compared with the pattern of the British diet, as calculated from two sources for the amino acid composition of foods. However calculated, the sulphur amino acids were the most limiting and the protein score was about 80.

The 'practical allowances' in the 1965 report for children and adolescents were lower, and for adults higher, than those in the 1957 report: applied to the UK population this resulted in an increase in the *per caput* allowance for reference protein of 6 g/day (Table 1). Comparison is made with British and American allowances, and with estimated available *per caput* UK protein supplies in 1964: 87 g/day (11% calories). While the 1965 report assumed 'that the score corresponds roughly

		NRC (1963)	FAO (1957)	FAO	(1965)
Daily per caput allowance (g) in terms of: Reference protein Dietary protein		6-	32	38 48*	
Available protein as % allowance ‡	72 101	61 122	40* 185*	156*	54† 136†
BMA, British Medical Association	ciation; N	IRC, National	Research Cour	ncil	

<sup>\*</sup>Using score of 80. †Using NPU of 70. ‡Allowing for a wastage of 15% at the 'retail' level of distribution.

to the NPU' it preferred the use of the latter: using a calculated value of 70 (NPU<sub>op</sub>; Miller & Payne, 1961) available supplies exceeded 1965 allowances by about one-third.

### REFERENCES

British Medical Association. (1950). Report of the Committee on Nutrition. London; British Medical Association.

FAO (1957). F.A.O. nutr. Stud. no. 16.

FAO (1965). F.A.O. nutr. Mtg Rep. Ser. no. 37.

Miller, D. S. & Payne, P. R. (1961). Br. J. Nutr. 15, 11.

National Research Council (1963). Publs natn. Res. Coun., Wash., no. 1146.

Vitamin A requirements of intensively fattened cattle. By J. H. Topps,\*

Department of Agriculture, University College of Rhodesia, Salisbury, Rhodesia,
and R. C. Elliott, P. D. Johnson and W. D. C. Reed, Henderson Research
Station, Mazoe, Rhodesia

Rates of depletion of liver stores of vitamin A are increased, and the animal's requirement for the vitamin is probably enhanced, by high intakes of digestible energy (Erwin, Gordon & Algeo, 1963; Quarterman & Mills, 1964), by either inadequate levels of vitamin E or high levels of nitrate in the diet (Hatfield, Smith, Neumann, Forbes, Garrigus & Ross, 1961; Jordan, Neumann, Smith, Zimmerman

<sup>\*</sup>Present address: Rowett Research Institute, Bucksburn, Aberdeen.

& Vatthaver, 1961), and by certain other dietary factors. Since some of these factors are relevant to conditions in Rhodesia the effects on growth and on liver stores of vitamin A of adding various levels of vitamins A and E to a high-concentrate diet fed to young steers were examined.

Forty-five steers weighing approximately 290 kg, were used, five steers on each treatment while five 'control' animals were slaughtered at the beginning of the trial, which lasted 18 weeks. The animals were offered *ad lib*. a high-concentrate diet principally composed of ground white maize grain, cob and husk (87.0%) and ground-nut cake (9.9%). Four levels of vitamin A, 0, 4, 8 and 12 million i.u. per ton of food, factorially combined with two levels of vitamin E, 0 and 2500 i.u. per ton of food, were used.

Neither additions of vitamin A nor vitamin E to the diet affected growth of the steers. All groups gained weight rapidly ( $1.63 \pm 0.23$  kg daily) and there were no visible signs of vitamin A deficiency.

Table 1. Mean concentration and mean total content of vitamin A in livers of steers fed on a high-concentrate diet to which were added various levels of vitamins A and E

Level of	Concentration (mg/100 g fresh weight)			Total content (mg)		
vitamin A (i.u./ton)	Without vitamin E	With vitamin E	Mean	Without vitamin E	With vitamin E	Mean
o 4 million 8 million	2·7 7·8 10·7	1·8 7·9 11·0	2·3 7·9 10·8	165 430 578	117 462 622	141 446 600
12 million Mean	14·2 8·9	14·7 8·9	14.5	754 482	912 528	833

The level of vitamin A added to the diet significantly affected both the concentration and the total reserve of vitamin A in the liver. To maintain the concentration of vitamin A in fresh liver at the same level as that found in the 'control' steers, 264 i.u. vitamin A/kg body-weight were needed daily. The maintenance of total hepatic reserves required 65 i.u. vitamin A/kg body-weight daily.

### REFERENCES

Erwin, E. S., Gordon, R. S. & Algeo, J. W. (1963). J. Anim. Sci. 22, 341.

Hatfield, E. E., Smith, G. S., Neumann, A. L., Forbes, R. M., Garrigus, U. S. & Ross, O. B. (1961). J. Anim. Sci. 20, 676.

Jordan, H. A., Neumann, A. L., Smith, G. S., Zimmerman, J. E. & Vatthaver, R. J. (1961). J. Anim. Sci. 20, 937.

Quarterman, J. & Mills, C. F. (1964). Proc. Nutr. Soc. 23, x.

Food requirements of African cattle for survival. By R. C. ELLIOTT, W. R. MILLS and W. D. C. REED, Henderson Research Station, Mazoe, Rhodesia, and J. H. Topps,\* Department of Agriculture, University College of Rhodesia, Salisbury, Rhodesia

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The coming of the rains may be delayed, with disastrous results, and the total fall may vary greatly in amount in large areas of Central and Southern Africa. This is especially true of the drier regions where large numbers of cattle may, and often do, die of starvation. The number of deaths is invariably greater in areas which are overstocked.

The problem of feeding sheep during prolonged drought has been studied in Australia, (Franklin, 1951), but little or no research has been carried out to define the minimal food requirements of cattle for survival.

Nine mature, non-pregnant Africander cows, weighing approximately 440 kg, were divided at random into three groups and given for 32 weeks three different amounts (1815, 2725 or 3630 g/head daily) of a diet composed of concentrates (77%), roughage (20%) and minerals (3%). The animals had access to both water and a salt lick. Digestibility of the diet was measured during the last week using chromic oxide as an indicator and found to be 78.3%.

One cow on the intermediate level of feeding died of bloat after 23 weeks whilst the remaining eight survived the trial. Depraved appetitite in the form of high intakes of salt and the chewing of fencing poles, and an increasing lethargy as the trial progressed, were obvious features of the animals' behaviour.

Mean losses of body-weight were approximately inversely proportional to amounts of food provided.

Table 1. Mean intake of food, initial body-weights and losses of body-weight over 32 weeks of groups of three Africander cows

Food offered (g/24 h)	Food intake (g/24 h)	Initial body-weight (kg)	Loss of body-weight (kg)			
1815	1815	440	149			
2725	2725†	443†	98†			
3630	3450	444	63			
†Mean for two animals.						

All groups lost considerable weight in the 1st week (40–60 kg/cow). Weight changes of all the animals except those of two receiving the largest amount of food could be accurately described by an exponential model. This statistical treatment allowed the estimation of asymptotic body-weights which corresponded to 'stability' of weight. At the two lower levels of feeding, maintenance of body-weight apparently occurred when intakes of digestible energy were less than recently published requirements (Agricultural Research Council, 1965).

### REFERENCES

Agricultural Research Council (1965). The Nutrient Requirements of Farm Livestock. No. 2, Ruminants. London: Agricultural Research Council. Franklin, M. C. (1951). Aust. vet. J. 27, 326.

The relationship between endogenous loss of magnesium and faecal output in the rat. By B. S. W. Smith (introduced by A. C. Field), Moredun Institute, Edinburgh, 9

Previous work has shown that the time taken for food to pass through the alimentary tract in the rat is inversely related to the faecal output (Smith, 1964). Also, rats on a restricted intake had a lower endogenous excretion of magnesium than animals fed *ad lib*. a similar magnesium-deficient diet in an earlier trial (Smith & Field, 1963). These findings suggested that magnesium excretion might be positively correlated with faecal output, as was found to be the case for nitrogen in rats (Mitchell, 1926) and for phosphorus in cows (Kleiber, Smith, Ralston & Black, 1951).

The effect of faecal output on endogenous magnesium excretion was examined by feeding three groups of adult rats a magnesium-deficient diet containing 0, 5 and 10% powdered cellulose (Whatman ashless powder). Each group of rats was made up of four females (average weight 358 g) and two males (average weight 455 g) housed in pairs of one sex. The diet (Smith & Field, 1963) which contained 5  $\mu$ g/g of magnesium was tableted to reduce wastage and facilitate separation of spilled diet and excreta.

The faeces and urine were collected separately and bulked over 3-day collection periods for 60 days. The faeces were dried to constant weight, the corresponding urine sample added and the total magnesium determined by analysis (Smith & Field, 1963).

There was no significant change in the faecal output of any of the rat pairs during the experimental period, showing that the magnesium-deficient diet did not affect the appetite. There was, however, a large increase in faecal dry matter due to the added cellulose. The mean daily faecal outputs for the animals receiving 0, 5 and 10% cellulose were 1·16, 2·82 and 3·98 g/kg body-weight respectively. Digestibilities were 96, 91 and 87% respectively.

No significant differences were found between the endogenous magesium excretions of any of the dietary groups or between sexes. The mean value for all groups during the period 7–36 days was 1.83 mg/kg body-weight day.

The magnesium excretion remained constant for 51 days. However, during the last 9 days of the experimental period the mean daily magnesium excretion dropped to 1·16 mg/kg body-weight. There was no corresponding reduction in faecal drymatter output. The significance of the reduction in magnesium excretion will be discussed.

I thank Mr J. L. Lyle of the Department of Pharmacy, Heriot-Watt University, Edinburgh for tableting the diets.

### REFERENCES

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Kleiber, M., Smith, A. H., Ralston, N. P. & Black, A. L. (1951). J. Nutr. 45, 253. Mitchell, H. H. (1926). Bull. natn. Res. Coun., Wash. no. 55. Smith, B. S. W. (1964). Proc. Nutr. Soc. 23, xxv. Smith, B. S. W. & Field, A. C. (1963). Br. J. Nutr. 17, 59.
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The thermic response to meals of varying size in the resting and active human subject. By M. J. Stock, Department of Nutrition, Queen Elizabeth College, London, W 8

Recent work by Miller & Mumford (1964), Brown, Miller & Mumford (1965), and Doyle, Morse, Gowan & Parsons (1965) on the effects of overfeeding human subjects would seem to indicate the existence of a homoeostatic mechanism for weight control. In this connexion Passmore & Ritchie (1957) have shown, after previous overfeeding, a greater increase in the rate of heat production in response to a standard meal than when previously fasted. A higher metabolic rate, however, is not necessarily an indication of a greater thermic response as the time course of the response must be allowed for.

During a recent overfeeding experiment we had the opportunity to make measurements of the total thermic response to meals of varying size. It was found that when the meal size was approximately doubled the thermic response was increased sixfold (mean result for six meals, two subjects). This sixfold increase, however, is equivalent to only 9% of the caloric value of the meal and will not account for the observed weight maintenance of these two subjects who had, on average, increased their daily intake by 68%. It was found, though, that estimates of the oxygen consumption of overeating subjects throughout a normally active day, indicated a 24 h heat production that matched daily caloric intake. This increase in heat production in response to increased intake cannot be accounted for in terms of greater activity per se (as measured by pedometer). This apparent paradox can be resolved by a further observation of ours which suggests a greater thermic response to a meal in the active state as compared to the resting. In eleven subjects receiving while resting meals supplying 1027 kcal postprandial heat production increased by 18 kcal/h, or 28% over BMR. By contrast, in seven subjects receiving meals of 1141 kcal, while exercising, the postprandial increase was 36 kcal/h, an increase of 56% over BMR. The exercise involved taking twelve steps/min each of 11 in during periods of 30 min. It appears from these results that besides the meal stimulating the metabolic rate, this stimulation is itself potentiated by an exercise-induced elevation of metabolism.

According to the work of Rubner (1902) on specific dynamic action, the thermic effect of food is related to the amount of protein ingested, while later workers (Forbes, Swift, Black & Kahlenberg, 1935; Miller & Payne, 1962; Kleiber, 1945-6) suggest that the effect is related to nutrient balance. However the thermic responses observed in this study show a direct relationship to the calorie value of the meal, which is in keeping with the conclusions of Brody (1945) with cattle maintained at different planes of nutrition.

This work is part of a study on overeating in collaboration with D. S. Miller and Pamela Mumford.

### REFERENCES

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Brody, S. (1945). Bioenergetics and Growth. New York: Hafner Publishing Company Inc. Brown, A. M., Miller, D. S. & Mumford, P. M. (1965). Proc. int. Congr. Diet. 1v. Stockholm, p.114. Doyle, M. D., Morse, L. M., Gowan, J. S. & Parsons, M. R. (1965). Am. J. clin. Nutr. 17, 367. Forbes, E. B., Swift, R. W., Black, H. & Kahlenberg, O. J. (1935). J. Nutr. 10, 461. Kleiber, M. (1945-6). Nutr. Abstr. Rev. 15, 207. Miller, D. S. & Mumford, P. M. (1964). Proc. Nutr. Soc. 23, xliii.
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Miller, D. S. & Payne, P. R. (1962). J. Nutr. 78, 255.

Passmore, R. & Ritchie, F. J. (1957). Br. J. Nutr. 11, 79.

Rubner, M. (1902). Die Gesetze des Energieverbrauchs bei der Ernährung. Leipzig und Wein: Deutiche.

The effect of a meal on the rate of ethanol metabolism in man. By D. S. Miller and J. L. Stirling, Department of Nutrition, Queen Elizabeth College, London, W 8

In preparation for the determination of body water by alcohol dilution in overeating subjects, we investigated the effect of a meal on the metabolic rate of alcohol. It is generally accepted that the decrease in concentration of alcohol in mammalian body fluids follows a rectilinear course from the time that absorption is ended and equilibrium throughout body fluids is established (Jacobsen, 1952; Pawan & Hoult, 1963). Factors influencing the rate of alcohol metabolism in man are of considerable interest both therapeutically and forensically, but substantial increases have not been effected with certainty by any treatment other than by fructose administration (Lundquist & Wolthers, 1958).

Using the method of Pawan & Hoult (1963) for the determination of body water we have shown that when a meal is fed to subjects during the alcohol postabsorptive period, there is a significant departure from linearity for the alcohol metabolism curve and that the maximum rate of metabolism is substantially increased. The experiment was carried out twice on six subjects, firstly under fasting conditions and secondly by feeding a 1000 kcal meal 1 h after the administration of the alcohol. Diuresis was maintained by a water load and alcohol was determined in the urine at half-hourly intervals. In all subjects the rate of alcohol metabolism increased postprandially. (Mean maximum fasting rate of alcohol metabolism  $81.5 \text{ mg/kg h} \pm 3.35 \text{ SE}$ ; mean maximum postprandial rate  $178 \text{ mg/kg h} \pm 11.9 \text{ SE}$ )

Since adequate time was allowed for the absorption of the alcohol, it is clear that the increased rate of metabolism of alcohol is due to metabolites from the meal. However the effect is considerably greater than that expected from the fructose (free or bound) content of the meal (Lundquist & Wolthers, 1958). The results are parallel to those of Stock (1966) and demonstrate that the consumption of a meal results not only in an increased overall metabolic rate but also in an increased rate of metabolism of an abnormal metabolite previously ingested. On the other hand the thermic effect of the meal is enhanced by exercise although it is reported that the rate of alcohol metabolism is not (Barnes, Cooke, King & Passmore, 1965). These authors also claim that alcohol has no specific dynamic action (SDA) but it may be that SDA affects alcohol metabolism. Certainly in the determination of body water by alcohol dilution the subjects should be fasting.

### REFERENCES

Barnes, E. W., Cooke, N. J., King, A. J. & Passmore, R. (1965). Br. J. Nutr. 19, 485. Jacobsen, E. (1952). Pharmac. Rev. 4, 107. Lundquist, F. & Wolthers, H. (1958). Acta Pharmac. tox. 14, 290. Pawan, G. L. S. & Hoult, W. H. (1963). Biochem. J. 87, 6P. Stock, M. J. (1966). Proc. Nutr. Soc. 25, xxxvii.

Delayed protein supplementation of barley diets for weanling pigs. By M. L. Yeo and A. G. Chamberlain, Department of Agriculture, University College of North Wales, Bangor

Nine castrated weanling pigs were given unsupplemented barley meal at the morning feed and an equal weight of a high-protein meal (23% crude protein) composed of barley, white-fish meal, vitamins and minerals during the afternoon. A control group of nine pigs were given the same total amounts of barley and high-protein meals, but as a mixture of equal parts at both feeds. The two groups of pigs were genetically similar.

Nitrogen balances were carried out in the 3rd and 6th week of the experiment. The apparent digestibility and percentage nitrogen retention did not differ significantly between collection periods or treatments. Growth rate and feed conversion between 15 and 55 kg live weight, and carcass leanness as judged by dissection, were also similar for both treatments.

These results support the findings of Eggert, Brinegar & Anderson (1953) who showed that weanling pigs can utilize the protein from maize and a high-protein supplement equally efficiently if the supplement is given at every other feed (24 h intervals) or at each of two daily feeds. They differ from those for rats where delayed supplementation of imbalanced protein diets often fails to restore growth rate to levels attained when balanced protein is fed (e.g. Geiger, 1947).

The application of these results to practical pig-feeding systems was discussed.

### REFERENCES

Eggert, R. G., Brinegar, M. J. & Anderson, C. R. (1953). J. Nutr. 50, 469. Geiger, E. (1947). J. Nutr. 34, 97.

### DEMONSTRATION

Determination of the oxygen consumption and the carbon dioxide production of the rat. By M. J. Stock, Department of Nutrition, Queen Elizabeth College, London, W 8

There have been numerous methods described for determining the metabolic rate of small mammals by indirect calorimetry. The techniques available generally suffer from one of several drawbacks, such as being too insensitive for short-term measurements, or involving intricate and/or expensive instrumentation. Frequently oxygen consumption only is measured, making the assessment of the respiratory quotient impossible.

The technique described here is similar in principle to that developed by Haldi, Wynn & Breding (1961) but involves the use of conventional apparatus only. It has the added advantage of allowing for the measurement of both the oxygen consumption and the carbon dioxide production over periods of time ranging from 2 to 18 h.

The metabolic chamber consists of a large dessicator, beneath the grid of which is installed a dish containing a 80% (w/v) solution of potassium hydroxide for the

absorption of the carbon dioxide produced. Moisture accumulating in the chamber is taken up by a layer of activated alumina pellets (Peter Spence & Sons Ltd) surrounding the dish of potassium hydroxide. The dessicator, containing the rat and filled with room air, is connected to a 6 l. spirometer filled with oxygen. As oxygen is consumed and the produced carbon dioxide is absorbed, oxygen enters the dessicator from the spirometer thus maintaining the composition of the air surrounding the rat. Oxygen consumed is read directly from the calibrated spirometer and carbon dioxide produced is determined by titration of the potassium hydroxide solution which is recovered quantitatively at the end of the experiment.

It is considered unnecessary to provide for temperature control as it was found that within 5 min of the rat being placed in the chamber the temperature had reached 26° and was maintained at this level which is close to that of our animal room (27°).

The method has proved to be rapid, reliable and capable of producing consistent results. Ten observations were made on a group of female rats (300 days old, average weight 300 g) maintained on stock diet (Amvilac no. 2). The measurements were made for periods of 2.5 h and the means of the ten observations were, for oxygen consumption 113 ml/100 g per h ( $SE = \pm 5.5$ ) and for the respiratory quotient 0.85 ( $SE = \pm 0.02$ ).

The period of measurement can be extended to 18 h by increasing the volume of potassium hydroxide used and refilling the spirometer halfway through the period.

### REFERENCE

Haldi, J., Wynn, W. & Breding, H. (1961). J. appl. Physiol. 16, 923.