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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Seventieth Meeting of The Nutrition Society was held at The Hannah Dairy Research Institute, Ayr, on Friday, 19 *February* 1965, *at* 1.45 pm, *when the following papers were read :*

The effect of diet on the growth of the cestode *Hymenolepis diminuta* in the intestine of the rat. By D. F. METTRICK*, C. A. HOPKINS and H. N. MUNRO, *Wellcome Laboratories for Experimental Parasitology and Institute of Biochemistry, University of Glasgow*

There is a considerable body of evidence to show that animals on low intakes of protein have a greater susceptibility to helminth infections and that the parasites flourish better on such diets (see review by Scrimshaw, 1964). On the other hand, some authors working with the cestode *Hymenolepis diminuta* (Chandler, 1943; Read, 1959) have observed a relationship between growth of the parasite and intake of carbohydrate but not of protein.

We have evaluated the effect of diet on the growth of *H. diminuta* in the rat. The animals were fed diets of constant caloric content but varying in quantity and quality of protein, quantity of carbohydrate and fat, and some were also given supplements of amino acids. When casein was removed from the diet and replaced with carbohydrate, the dry weight and nitrogen content of the parasite was markedly increased. However, replacement of casein by fat had no effect on worm development, indicating that removal of protein is not a significant factor, whereas addition of carbohydrate to the diet is effective. Since most previous investigators made up protein-deficient diets by replacing protein with carbohydrate, it seems probable that the additional carbohydrate may have contributed to the increased worm growth frequently observed by them.

Exchange of zein for casein in the diet failed to influence parasitic growth, and addition of tryptophan and lysine to the meal containing zein was also without significant effect. These findings indicate that growth of *H. diminuta* is uninfluenced by quality of dietary protein. However, when tryptophan and lysine were fed to the host at times other than those of giving zein, parasitic growth was considerably depressed. This effect is unrelated to the quality of protein fed in the rest of the diet, since the depression in worm growth can be demonstrated by their addition to a protein-free diet rich in carbohydrate. Addition of these amino acids singly, either to diets containing protein or deficient in protein, had significant actions on worm growth.

One of us (D.F.M.) gratefully acknowledges the award of a Commonwealth Bursary from the Royal Society and the Nuffield Foundation.

*On leave of absence from the University of the West Indies.

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Methane production of ruminants given natural feeds. By J. L. CLAPPERTON and K. L. BLAXTER, *Hannah Dairy Research Institute, Ayr*

The results of 2286 24-h determinations of methane production by cattle and sheep made during the course of other experiments have been analysed statistically. Analytical error was shown to be less than $\pm 3\%$ of the amount of CH_4 produced. The day-to-day variation of CH_4 production was $\pm 7{\cdot}2\%$ of the amount produced in both sheep and cattle. With sheep the between-animal variation was found to be $\pm 7{\cdot}2\%$ and $\pm 8{\cdot}1\%$ of the amount produced in two separate series of experiments.

Experiments with forty-eight different diets were included in the analysis. With each of these diets CH_4 production fell when the amount of diet given was increased. For convenience the effect of diet on CH_4 production was expressed, firstly as the amount produced at the maintenance level of nutrition and, secondly, as the change in production which occurred on doubling feed intake to $2\times$ the maintenance level. The regression of CH_4 production at maintenance, C, (kcal CH_4/100 kcal feed ingested) on the apparent digestibility of feed at maintenance, D, (kcal apparently digested/100 kcal feed ingested) was

$$C = 3{\cdot}67 + 0{\cdot}062\ D.$$

The residual standard deviation was $\pm 0{\cdot}34$ kcal CH_4/100 kcal feed and the regression was statistically significant when $P < 0{\cdot}001$. At the maintenance level of feeding CH_4 production thus increased from 6·5 to 8·9 kcal/100 kcal feed as the apparent digestibility of feed increased from 45 to 85%.

The change in CH_4 production on increasing the amount of feed from maintenance to $2\times$ maintenance ($\triangle C$) was also related to apparent digestibility measured at the maintenance level. The equation, statistically significant when $P < 0{\cdot}001$, was

$$\triangle C = 2{\cdot}37 - 0{\cdot}050\ D,$$

with a residual standard deviation of $\pm 0{\cdot}65$ kcal CH_4/100 kcal feed.

Combining these equations shows that while at maintenance levels of nutrition, CH_4 production expressed as kcal CH_4/100 kcal feed tends to rise with increased apparent digestibility, at levels of feeding greater than $2{\cdot}2\times$ maintenance it tends to fall. The results show that irrespective of feeding level with diets with an apparent digestibility of 40% 15–16% of the energy apparently digested is lost as CH_4. When apparent digestibility is 60%, about 12% of the energy apparently digested is lost as CH_4. When apparent digestibility is 80%, 8–11% of the energy apparently digested is lost as CH_4, the lower values occurring at the higher feeding levels.

Methane production from formic acid. By J. E. VERCOE and K. L. BLAXTER, *Hannah Dairy Research Institute, Ayr*

Carroll & Hungate (1955) among others showed by experiments in vitro that formic acid was rapidly dissimilated by rumen micro-organisms to form methane and carbon dioxide according to the equation

$$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O.$$

Reid (1962) concluded, however, that formic acid was absorbed by the ruminant in appreciable quantities and cited experiments which showed that infusion of formic acid into the rumen markedly reduced the heat production of the animal.

Five experiments were made in which 1·0 or 1·5 moles of formic acid were continuously infused for periods of 12–17 days into the rumens of fistulated sheep confined in respiration chambers. The gaseous exchange was measured each day and also during periods of up to 14 days before and after the infusions. The mean results of the experiments showed that CH_4 production increased by $4{\cdot}9 \pm 0{\cdot}5$ l. and CO_2 production by $18{\cdot}1 \pm 2{\cdot}8$ l. per mole formic acid given. A small increase in O_2 consumption occurred which statistically was not significant. These results do not differ significantly from those expected from the above equation for bacterial dissimilation of formic acid ($+5{\cdot}6$ l. $CH_4 + 16{\cdot}8$ l. CO_2, 0·0 l. O_2/mole formic acid). Infusion of 0·5 mole sodium formate into the blood in similar experiments did not result in significant changes in the respiratory exchange, an increase in urinary excretion of formate occurring.

In short-term experiments lasting 8–12 h, single doses of sodium formate (0·75, 1·0, 1·5 and 2·0 moles) resulted in increases in CH_4 production which were 89, 75, 73 and 59% of expectation. In all but one experiment, however, CO_2 production and O_2 consumption were depressed. These results suggest that rapid infusion of formate unlike long-term continuous infusion depresses the utilization of the basal diet.

It was concluded that under normal conditions of feeding, formic acid arising from bacterial metabolism is converted quantitatively to CH_4 and CO_2 and if any is absorbed it is unlikely to produce the marked depression of metabolism noted by Reid (1962).

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The effects of C_{18} acids on methane production by sheep. By J. W. CZERKAWSKI, K. L. BLAXTER and F. W. WAINMAN, *Hannah Dairy Research Institute, Ayr*

In the course of experiments with formic acid (Vercoe & Blaxter, 1965) it was noted that the concentration of C_{18} unsaturated acids in rumen liquor declined during the infusion of formic acid suggesting that the well-established hydrogenation of long-chain unsaturated fatty acids in the rumen (Garton, Lough & Vioque, 1961) might be related to methanogenesis. The experiments of Williams, Hoernicke, Waldo, Flatt & Allison (1963) which suggest that the carbon dioxide reduced to

methane in the rumen has the same specific activity as the CO_2 of the bicarbonate pool in the rumen also suggest that hydrogenation might be relatively non-specific.

Experiments have therefore been made in which crude linolenic acid (three experiments), oleic acid (two experiments) or linoleic acid (one experiment) has been infused into the rumens of sheep for periods of up to 24 days. Preliminary and final control periods of up to 20 days were included. The energy exchanges of the sheep were measured by indirect calorimetry.

Infusion of 88 g crude linolenic acid/24 h (60% linolenic, 14% linoleic, 16% oleic, 4% stearic and 6% palmitic) resulted in a depression of CH_4 production from 32·4 to 16·9 l./24 h. An increase in the amount infused to 128 g/24 h reduced CH_4 production to 12·2 l./24 h. Faecal losses of dry matter and of energy increased only slightly. When 88 g (820 kcal/24 h) were given the increase in the heat of combustion of the faeces was 58 kcal/24 h and with 128 g (1166 kcal/24 h) the increase was 101 kcal/24 h. When 78 g of linoleic acid were infused CH_4 production fell from 28·1 to 16·2 l./24 h; when 74 g oleic were infused CH_4 production fell from 29·7 to 19·5 l. in one sheep. In another given 72 g oleic acid, CH_4 production fell from 27·1 to 16·5 l./24 h. In no experiments was any marked change in the faecal loss of energy or of cellulose noted suggesting that despite a gross interference with methanogenesis, bacterial dissimilation of cellulose or other fibrous constituents was little affected.

Because the depression of methane production caused by a C_{18} acid is not directly proportional to its unsaturation, it appears that these acids as a class have an effect on methanogenic bacteria quite apart from any role they have as hydrogen acceptors. An effect of unsaturation can, however, still be discerned, amounting to a depression of methane production of approximately 0·33 moles CH_4/mole double bond supplied. These experiments are continuing.

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Estimation of lignin. By J. W. CZERKAWSKI, *Hannah Dairy Research Institute, Ayr*

Existing methods for the determination of lignin are laborious and involve large and variable corrections for the residual nitrogenous impurities (Armitage, Ashworth & Ferguson, 1948). An attempt was made to reduce the magnitude of these corrections while retaining the conventional 72% sulphuric acid isolation procedure. Both dried grass and sheep faeces have been examined and the method of Ellis, Matrone & Maynard (1946) as modified by Waite, Johnston & Armstrong (1964) was used as a starting point.

Incubation of grass with pepsin (50 mg/g grass in 50 ml 0·02 N-HCl) removed relatively little nitrogen. A subsequent digestion with pancreatin (100 mg/g grass in 50 ml borate buffer pH 8·6) removed considerably more nitrogen. Addition of

sodium dodecyl sulphate (final concentration 0·01%) which potentiates the activity of some pancreatic enzymes (Hall & Czerkawski, 1961) resulted in a greater reduction of nitrogen content than did pancreatin alone. Pepsin digestion appeared essential to ensure maximal removal of nitrogen by pancreatin. The nitrogen content of crude lignin isolated after complete treatment was 1–2% (cf. Bondi & Meyer, 1948). By the Ellis, Matrone & Maynard method the nitrogen content was 3–5%.

The lignin isolated in the above way from both feed and faeces was hydrolysed in 6 N-HCl at 105° in sealed tubes and the partial hydrolysate analysed by the Moore and Stein procedure by Dr W. Manson. Although complete recovery of nitrogen as amino acid was not attained, it was evident that at least 60% of the nitrogen of the lignin was present as amino acid nitrogen. The distribution of the amino acids in lignin isolated from grass was broadly similar to the distribution of amino acids in grass protein. The same was true of the distribution of amino acids in faecal lignin but, in addition, substantially larger amounts of lysine, valine and aspartic acid were found. These could have arisen from contamination of the faecal lignin by bacteria. It is concluded that the nitrogen content of lignin isolated by the 72% sulphuric acid procedure can be reduced by pancreatin treatment but even so it is largely, if not entirely, the result of contamination with protein of plant origin. The correction factor of $N \times 6{\cdot}25$ usually applied to isolated lignins is thus justified.

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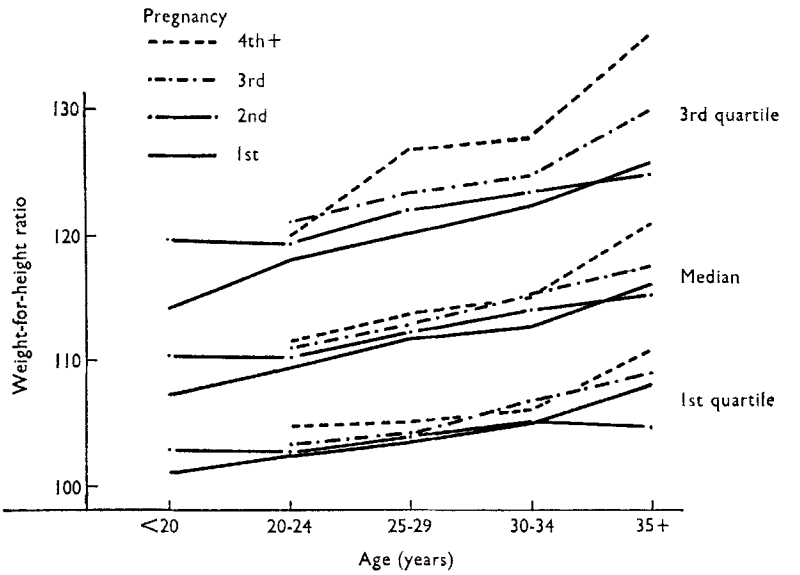
Maternal weight for height. By A. M. THOMSON and W. Z. BILLEWICZ, *MRC Obstetric Medicine Research Unit, Aberdeen Maternity Hospital*

Thomson & Hytten (1961) and Taggart (1961) have shown that the average woman lays down a good deal of fat during pregnancy. If this is not lost, body-weight should tend to increase with parity. Aberdeen Maternity Hospital records have been used to investigate trends with age and parity in maternal weight-for-height. Weight gain during the first 20 weeks of pregnancy (about 4 kg) is little influenced by maternal characteristics. Weight-for-height is expressed as the percentage of observed weight at 20 weeks to the standard weight of a non-pregnant woman of the same height (Kemsley, Billewicz & Thomson, 1962).

The results are summarized in the figure. Median weight-for-height ratios show a steady increase with age, equivalent to an average gain of some 8 lb from ages under 20 to ages over 35. Similar trends with age are shown by the upper and lower quartiles. Parity makes little difference to the medians and the lower quartiles, women having a fourth or later pregnancy being only about 2 lb heavier than primigravidae of the same age. The upper quartiles show a greater trend with parity. The distributions differ little by social class. There are, however, slightly more grossly overweight multiparae in the semi- and un-skilled working classes than in the



professional and managerial classes. It appears, then, that age is the main factor in determining maternal body-weight, but increasing parity does give rise to a minority of unusually heavy women. This is consistent with Sheldon's (1949) suggestion that women may 'at times' develop a severe obesity after having a baby.

While pregnant women tend to lay down fat, there appear to be compensating mechanisms whereby most of them lose this extra fat between pregnancies.

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Vitamin A utilization and protein level and quality. By J. QUARTERMAN and GABRIELA C. SARAIVA*, *Rowett Research Institute, Bucksburn, Aberdeen*

Rechcigl, Berger, Loosli & Williams (1962) have attempted to find a quantitative relationship between weight gain and vitamin A utilization in rats. In their experiments the rats were given an oral dose of either 0·6 or 0·4 mg vitamin A acetate (S. Berger, personal communication); in ours 1·0 mg. In the latter, control groups of rats were killed 1 day after dosing and the remaining groups were given various diets, free of vitamin A and carotene, for 3 weeks before killing. Initial age and sex of the rats differed in different experiments. Vitamin A was estimated in the lungs, kidneys and livers. The loss of vitamin A on a given diet during 3 weeks is the difference

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between the mean tissue vitamin A (total μg) of the control group killed at the beginning of the experiment less that of the group given the diet.

The table gives results of experiments in which the protein and amino acid components (replacing starch) were varied and the loss of vitamin A is calculated as a percentage of the total vitamin A in the control group.

Dietary protein	Mean wt gain (g) and SE	% loss of vitamin A and SE
None	−10·6± 1·1	11·2±2·1
Casein 9%	15·0± 5·4	27·5±8·4
Casein 9%	30·1±10·6	25·0±7·7
Casein 9%	90·0± 3·4	37·0±3·7
Casein 18%	78·0± 7·5	31·0±3·0
Casein 23%	114·0± 1·5	38·0±2·6
Gelatin 9%	−8·9± 1·2	20·0±3·2
Gelatin 18%	−10·0± 0·9	26·5±2·5

Dietary protein	Mean wt gain (g) and SE	% loss of vitamin A and SE
Gelatin 26%	44·0±7·0	47·0±22·0
Casein 9% (restricted feeding)	11·3±3·6	0·0± 7·7
Casein 18% (restricted feeding)	33·6±5·2	0·0±12·8
Casein 9% + glutamate 2%	24·4±3·2	21·0± 4·5
Casein 18% + glutamate 2%	64·3±2·0	25·0± 7·4
Casein 9% + lysine 2%	20·8±6·3	17·5± 5·5
Soya-bean diet, 23% crude protein	134±6·7	47·0± 2·4

From these results and those of Rechcigl *et al.* (1962) the following conclusions may be drawn. The relationship between the total amount (μg) of vitamin A lost and the weight gain during the experimental period is influenced by sex and by the initial level of the vitamin in the body. If the loss is calculated as the percentage figure a close proportionality to weight gain is observed, apparently independent of sex and the magnitude of initial stores.

This relationship did not hold where high levels of poor-quality protein were fed, when there was little growth and a high loss of vitamin A. The addition of 2% sodium glutamate or lysine to a casein diet had no effect on vitamin A loss. Restricted feeding of a high-quality protein diet caused unusually low loss of vitamin A.

The lungs had only about 0·8 and 2·3% and the kidneys about 0·4 and 1·1% as much vitamin A as the liver in the experimental and control groups respectively.

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The production and signs of zinc deficiency in the sheep. By C. F. Mills, J. Quarterman, R. B. Williams and A. C. Dalgarno, *Rowett Research Institute, Bucksburn, Aberdeen*

There are indications that zinc deficiency in ruminants may be more common than hitherto suspected. The aims of the work described here are to characterize the signs of zinc deficiency in the sheep, to establish the sequence of events during the development of zinc deficiency and to determine the most suitable criteria for the diagnosis of this deficiency.

A diet of low zinc content was made up of dried egg albumen, 6%; urea, 3%; cotton hull fibre, 9%; glucose, 32%; starch, 38%; arachis oil, 6% and mineral and vitamin supplements. The mineral supplement provided 0·6% of supplementary

calcium. The zinc content of batches of this diet was within the range 1–3 μg/g. Control animals were fed the same diet supplemented with zinc sulphate to give a final zinc content of 70 μg/g.

Ten 3-week-old Dorset Horn lambs were offered the low-zinc diet *ad lib.* with 3 pints of cow's milk daily for 6 weeks. Five lambs were subsequently given the zinc-supplemented diet on which they maintained rapid growth gaining weight at a rate of 2·1 kg/week when fed *ad lib.* Five other lambs were fed the low-zinc diet and within 7 days a retardation of growth was observed. After 14 days weight gain had ceased, food intake was depressed and excessive salivation and hyperkeratosis and pallor of the tongue were observed. Wool growth was retarded and hair was lost from around the mouth and eyes after 21 days. Parakeratotic lesions developed around the hocks and hooves by 28–35 days. Plasma zinc concentrations fell from 1·01 ± 0·08 μg/ml before depletion to 0·80 ± 0·09 μg/ml after 3 days and 0·22 ± 0·04 μg/ml after 9 days on the low-zinc diet.

A remarkably rapid response occurred when animals in this state of depletion were given 20 mg zinc orally (as $ZnSO_4$) each day. Within 24 h salivation had ceased and within 48 h wool growth resumed, food intake increased and the tongue regained its normal colour. All external lesions healed within 28 days.

Riboflavine and fat in cow milk. By E. C. OWEN and R. PROUDFOOT, *Biochemistry Department, Hannah Dairy Research Institute, Ayr*

Six Ayrshire cows of comparable stage of lactation each ate twice daily 8 lb cubes (oats : beans : flaked maize, 6 : 5 : 1 by weight) and 8 lb hay. Their milk yields ranged from 5–3 gal daily initially to 4–3 gal finally. Analyses on alternate days of each of three consecutive 3-week periods revealed the linear regression: fat % = 2·5 + 0·94 × riboflavine ppm (Fig. 1) thus recalling a much earlier report by Whitnah, Kunerth & Kramer (1938). The proportion of milk fat arising from acetic acid is still problematic (Lascelles, Hardwick, Linzell & Mepham, 1964). Riboflavine enzymes (Green & Gibson, 1960) are probably involved in the production of volatile

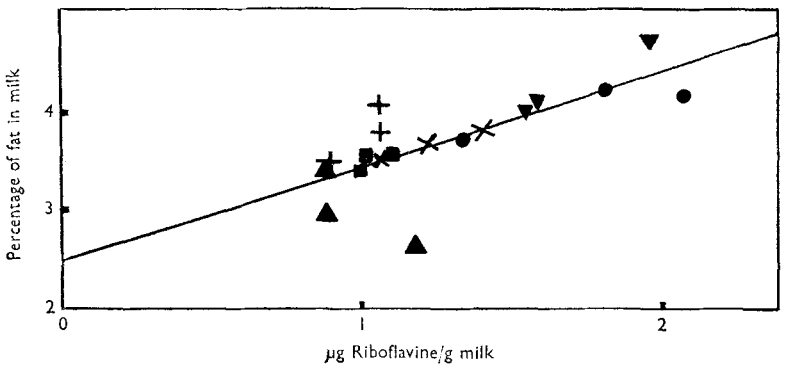

Fig. 1. Regression of fat percentage on concentration of riboflavine in milk of six cows (■, ▼, ▲, +, ×, ●) in three consecutive periods ($r = +0{\cdot}7276$; $P < 0{\cdot}001$).

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fatty acids by rumen bacteria and in their synthesis to higher fatty acids by the ruminant mammary gland. If this is so, it is possible that the 2·5 in the above regression represents physiological fat and that 0·94 × riboflavine represents fat arising from acetic acid of microbial origin. A partial test of this hypothesis is provided by our experiments (Chanda, McNaught & Owen, 1952) in which thyroxine, which causes translocation of body fat into milk, did not affect riboflavine in milk (Modi, Owen & Darroch, 1957) but caused an increase from 4·14 to 5·24 in its fat percentage, each figure being a 3-week average.

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Comparison of slow and rapid recovery in the sheep following induction of rachitic symptoms. By D. BENZIE and J. C. GILL, *Rowett Research Institute, Bucksburn, Aberdeen*

Previous experiments have shown that rickets can be induced in young, housed sheep by feeding them a diet adequate in calcium but low in phosphorus and unsupplemented with vitamin D. Restitution of phosphorus to adequate levels and injection of 10^6 i.u. of vitamin D_2 or D_3 intramuscularly caused ossification of the epiphysial cartilage and near-fusion of the epiphysis with the diaphysis of the radius and ulna to take place within a period of 6 weeks (Benzie, Boyne, Dalgarno, Duckworth, Hill & Walker, 1960; McRoberts, 1961; Duckworth, Benzie, Cresswell, Hill & Boyne, 1962). There was little or no bone accretion or remodelling of bone, although there was a slight increase in bone length during the period of repair.

In the present experiment rachitic symptoms were induced in each of five housed sheep by again feeding the same kind of diet but unsupplemented with vitamin D. The percentage composition of the concentrate fed consisted of 70% sugar-beet pulp, 12% blood meal, 8% sugar, 7·5% maize starch, 1% finely ground limestone, 1% salt, and 0·5% vitamin A powder. The vitamin A powder contained 540 000 i.u./lb. The daily allowance per animal was 500 g of this diet plus 25 g oat straw. This was estimated to contain adequate protein to meet the requirements of normal growth and give sufficient energy to keep the animals in moderate condition. The average daily intake of each animal was 3·5 g Ca and 0·34 g P.

After a year on this diet the sheep were grazed out-of-doors on good pasture without receiving vitamin D supplementation, and good recovery of bone disturbances took place.

In comparison with recovery following supplementation with vitamin D as in the previous experiment, the ossification of the epiphysial cartilage was much slower, bone accretion was marked, bone length was considerably greater, and remodelling of bone more complete. Radiographic reproductions will be demonstrated.

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The nutritive value of leaf protein concentrates. By A. A. WOODHAM, *Rowett Research Institute, Bucksburn, Aberdeen*

Results already published (Duckworth & Woodham, 1961), showed that samples of leaf protein concentrate (LPC) prepared from five types of green leaves by a process involving acetone extraction were all high in nutritive value and approximately equivalent to soya-bean meal as a supplementary protein source in chick diets. Work on rats (Henry, 1964), has in general confirmed this, though low values were obtained for samples of protein prepared from tares and sweet clover. The purpose of this communication is to present some additional results obtained in this laboratory using a chick GPV method (Duckworth, Woodham & McDonald, 1961), a chick PER method (Bunyan & Woodham, 1964), and a rat PER method (Duckworth & Woodham, 1961). In addition chemically determined available lysine values (Carpenter, 1960), are reported for three samples.

Biological and chemical evaluation of various leaf protein concentrates

		1961			1963		
Source of concentrate	1959 GPV	GPV	PER Chick	PER Rat	GPV	PER Chick	ALV (g/16 g N)
Wheat		61	1·67	1·84	85	2·18	5·22
Tares	82	72	1·78	1·76			
Rye	82				75	2·06	5·04
Barley	77						
Kale	75						
Mixed grasses	71						
Pea		51	1·49	1·83	68	1·90	5·21
Maize					64		
Clover		54	1·35	1·58			
Mustard		50	1·35	1·56			

All samples used were prepared at Rothamsted by Mr N. W. Pirie, FRS, but while those examined in 1959 had been extracted and dried there using a technique which involved acetone extraction, the samples examined in 1961 and 1963 were transported to Aberdeen in the wet frozen state and then dried by a commercial accelerated freeze-drying (AFD) process (Gooding & Rolfe, 1955).

The additional results confirm the potentially high value of proteins from leaves but there are indications that different batches of protein from the same plant may vary in quality, and some comparatively poor samples—notably from clover and mustard—have been encountered.

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The One Hundred and Seventy-third Meeting of The Nutrition Society was held at the National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 on Saturday, 22 May 1965, *at* 10.15 *am, when the following papers were read:*

Rumen volatile fatty acid proportions and herbage diets. By D. J. THOMSON and R. A. TERRY, *Grassland Research Institute, Hurley, Maidenhead, Berks.*

Areas of a sward of S.23 perennial ryegrass were differentially fertilized with nitrogenous fertilizer, harvested on dates on which previous studies had indicated the herbages would be of similar digestibility, and barn-dried.

The chemical composition of the herbage feeds was (% dry matter):

	Soluble carbohydrate	Nitrogen	% apparent digestibility of energy
Feed 1	20·2	2·8	78·5
Feed 2	6·6	4·7	77·9

Lambs fitted with rumen cannula were used to study the rumen volatile fatty acid patterns produced by these two herbage feeds. The feed was offered once per day for 14 days, and on days 12 and 14 the rumen was sampled six times between 10.30 am and 4.30 pm. Volatile fatty acids were determined on a bulked sample. The proportions given in Table 1 are means from three lambs each sampled on 2 days.

Table 1. *Molar percentages of acid*

	Acetic	Propionic	Butyric	pH
Feed 1	60·6	26·8	12·6	5·89
Feed 2	67·0	21·4	11·6	6·35
SE of mean	±0·58	±0·34	±0·37	±0·070

Statistically significant ($P=0·05$) differences between the feeds were found in the proportions of acetic and propionic acids in the rumen, but not in those of butyric acid.

In this experiment the rumen was sampled during only 6 h of the 24. The possible magnitude of the diurnal variation was examined by rumen-sampling one lamb throughout a 24 h period (Table 2).

Both experiments demonstrated the difference between the rumen acid patterns produced by these two herbage feeds, but also showed the necessity of comprehensive sampling of the rumen to avoid misleading results.

Table 2. *Molar percentages of volatile fatty acids*

		Sampling times								
Acid	Feed	10.30	11.45	12.45	14.30	16.30	20.00	24.00	6·30	10·30
Acetic	1	64·9	60·5	59·1	59·3	60·6	60·1	63·2	67·9	68·1
	2	68·4	71·2	69·2	71·9	70·1	69·7	69·2	68·4	68·9
Propionic	1	24·0	28·5	29·5	28·9	28·2	27·4	26·2	22·7	21·6
	2	18·9	19·5	19·9	18·9	20·7	21·0	21·1	20·2	18·9
Butyric	1	11·1	11·0	11·5	11·8	11·2	12·5	10·7	9·4	10·3
	2	12·7	9·4	10·9	9·2	9·2	9·4	9·6	11·5	12·3

The effect of varying rumen volatile fatty acid proportions on the energy retention and carcass composition of lambs. By D. J. THOMSON, *Grassland Research Institute, Hurley, Maidenhead, Berks.*

Equal quantities of digestible energy of each of the two herbage feeds described in the previous communication were fed at two planes of nutrition (in the ratio 100 : 70) to growing worm-free lambs, housed indoors and fed individually once per day. An initial group of sixteen lambs was slaughtered at the beginning of the experiment and the experimental groups, each of eight lambs, were slaughtered at the end of an 82-day feeding period. Gross energy, fat, nitrogen, ash and moisture determinations were made on the carcass, offal, wool, skin and blood of all lambs.

The carcass energy, fat and protein of each experimental lamb was estimated from regressions based on the initial slaughter group and subtracted from the final values to give the energy, fat and protein stored in the carcass.

Table

Feed	Plane of nutrition	Carcass energy retention (Mcal)	Fat retention (g)	Protein retention (g)
1	Low	18·69	1605	712
	High	32·90	2918	1060
2	Low	21·00	1766	826
	High	31·26	2617	1175
SE of mean of eight		±1·367	±141·8	±52·1

The final carcass weight at each plane of nutrition was the same on both feeds. Carcass energy and fat retentions were similar, but the lambs on feed 2 stored significantly ($P=0·05$) more protein in the carcass than those on feed 1.

Interpretation of the results cannot be made solely in terms of differences in rumen volatile fatty acids but must also take account of the different levels of nitrogen intake.

Observations on the interaction of protein and calcium during reproduction in the rat. By N. R. H. EL-MARAGHI and R. J. C. STEWART, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW7*

The importance of a balanced intake of protein and calcium for the growth and

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maintenance of normal bone has been demonstrated in young, adult and aged rats (El-Maraghi, 1964; El-Maraghi & Stewart, 1963, 1964).

The table shows the effects on the mother and offspring of dietary protein and Ca during gestation and lactation.

Some effects of diets with different protein and calcium contents on the bones of rats and their offspring

			Individual offspring					Femurs of mothers			
			Weight		Ash			End 2nd lactation			
Diet of mother		Total pups in six litters	0 days (g)	21 days (g)	0 days (mg)	21 days (mg)	Ash/cm³ femur 21 days (mg)	Total ash (mg)	A/R	mg ash/cm³	End 1st gestation (mg ash/cm³)
NDpCal%	Ca (%)										
10·2	0·44	58	5·4	32	58	840	153	174	1·58	464	663
5·1	0·44	48*	4·7		54						606
10·2	0·11	66	5·3	30	53	656	114	93	1·02	241	492
5·1	0·11	49	5·1	15	54	376	132	91	1·31	286	577

*Only three pups survived beyond 1 week of age.

The protein-calorie deficient mothers produced fewer and slightly smaller young than did the normal rats and, when the intake of protein was low and of Ca high, postnatal losses were high. At birth the average ash content of the young ranged from 53 to 58 mg and at weaning (21 days) had risen to 376—840 mg. These variations could be related to growth, the rats suckled by protein-calorie deficient mothers being only half the weight of the controls. Ash/cm³ of the femur varied from 114 to 153 mg being high in the high-protein, high-Ca and low in the high-protein, low-Ca group. A single pregnancy (no lactation) did not have a serious effect on the mineral content of the bones of the mothers, but a second pregnancy and lactation led to a great reduction in the total ash, ash/cm³ and, especially when the Ca intake was inadequate, the A/R ratio of the bone.

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Diet methodology: a comparison between a 3-day and a 7-day study on 200 infants. By J. V. G. A. Durnin, W. J. McLees and C. C. Macleod, *Institute of Physiology, University of Glasgow*

Malnutrition in Glasgow children, 1964. By G. C. Arneil (introduced by D. P. Cuthbertson), *Department of Child Health, University of Glasgow*

Ready availability of expert nutritional advice is no guarantee of its adequate application. From November 1963 until June 1964, three surveys were carried out in the child population of Glasgow.

The first survey was of 400 children, 205 from a poor residential area and 195 from a moderate area. These children were selected by random numerical sampling of birth date. Detailed dietary histories were taken and particular emphasis placed on vitamin D-containing foods and supplements. The results indicate clearly that breast feeding is rapidly receding (only 5% of babies were fed to 8 weeks). The use of unfortified liquid milk is increasing from an early age, probably due to the ease of obtaining and feeding it. The mothers of the final 300 subjects were asked to bring the child to hospital and 101 did so. Of these seventy-six agreed to venepuncture and radiological examination. Three of these seventy-six children had active rickets. Furthermore 32% had a haemoglobin level below 11 g/100 ml and two children levels below 9 g/100 ml.

In the second survey a slum area was investigated and the fourth and later children (aged 12–36 months) of poor homes investigated by clinical examination, X-ray of wrist and haemoglobin estimation. One hundred children were investigated and of these two had active rickets. In this group 59% had a haemoglobin level of less than 11 g/100 ml, 9% less than 9 g/100 ml and 3% less than 7 g/100 ml.

The third survey of the presentation of infantile rickets at the Royal Hospital for Sick Children, Glasgow gave clear evidence of a rise in infantile rickets from eleven cases during the 6-year period 1953–8 to forty-eight cases during the 3-year period 1962–4. The rise is in 'white' children.

The treatment of obesity with a bread of high protein and low carbohydrate content. By A. N. HOWARD, *Department of Pathology, University of Cambridge* and T. B. ANDERSON, 1 *Huntingdon Road, Cambridge*

Diets for the treatment of obesity can be effective even when the patient is allowed unrestricted amounts of protein and fat, providing the carbohydrate intake is kept low. Because of the high cost of animal protein and the unpalatability of excessive amounts of fat, many patients find it difficult to adhere to such diets. To provide an inexpensive source of protein, a highly palatable dietetic bread made from wheat germ and soya flour has been developed containing 25% protein, 7% fat, 15·7% carbohydrate, 0·45% salt, 40% moisture and 233 kcal/100 g (Spillers Limited, Cambridge). The effectiveness of this product in helping patients adhere to a diet of high protein and low carbohydrate content has been examined.

Seventy-two patients under the care of twelve general practitioners were divided into two equal groups evenly matched with respect to doctor, sex, age, percentage overweight, height, intelligence and previous history of obesity. Patients were instructed to follow for 8 weeks, a slimming diet in which table salt and foods of high carbohydrate content were forbidden. Foods containing chiefly protein and fat were unrestricted, but others with a moderate carbohydrate content were allowed in small amounts. The diet was supplemented by 150 g/day bread supplying approximately 350 kcal. One group was given the dietetic bread described above, the other a commercial germ bread (9·8% protein, 1·8% fat, 46% carbohydrate, 0·45% salt, 40% moisture and 260 kcal/100 g).

After 8 weeks, for patients on the dietetic bread, the mean weight loss was 7·1 lb, 89% lost more than 2 lb and 84% patients reported their hunger was completely satisfied. For those given the commercial germ bread, the mean weight loss was 5·3 lb ($P < 0{\cdot}05$; Mann-Whitney U-test) 72% lost more than 2 lb and 63% reported their hunger was completely satisfied. Calculations from records of food consumption by patients in both groups showed that approximately 1500 kcal were consumed daily.

Experiments over a longer period are in progress to determine if the treatment of obesity using a diet of high protein and low carbohydrate content can be successful, and if a dietetic bread of high vegetable protein content can help to satisfy appetite.

Acknowledgements are made to Spillers Limited, Cambridge, for generous supplies of bread and to Drs H. Cornford, S. H. Gould, A. J. Haines, K. B. Hallam, G. A. Hart, B. Reiss, C. A. Sills, N. Silverston, G. F. Swan, C. W. Walker and R. Salisbury Woods, Cambridge, for their collaboration.

Tissue phospholipids in hypervitaminosis A. By U. K. Misra (introduced by I. Macdonald), *Department of Radioisotopes and Biochemistry, V. P. Chest Institute, University of Delhi, Delhi-7, India*

Plasma phospholipid levels have been reported to increase in humans and rats given excessive doses of retinol (Van Bruggen & Straumfjord, 1948; Misra, 1965). Increased incorporation of $^{32}P_i$ (radioactive inorganic phosphate) has also been reported in to plasma phospholipids of hypervitaminotic A rats (Misra, 1965). The role of retinol has been implicated to control the permeability of biological membranes (Lucy & Dingle, 1962). This communication describes the effect of hypervitaminosis A on tissue phospholipids of rats.

Retinol, 100 000 i.u., was given intramuscularly daily for 10 days to male rats (145–150 g). On the 10th day rats in control (no retinol) and experimental groups were intraperitoneally given 100 μc/100 g body-weight $NaH_2{}^{32}PO_4$. Rats were sacrificed after 6 h and liver, kidney, and small intestine were removed, cleaned, weighed and lipids extracted with chloroform : methanol (Folch, Lees & Stanley, 1957). Lipid extracts were analysed for phosphorus (Bartlett, 1959) and ^{32}P activity. Tissue phospholipids were separated on silicic acid papers and phospholipid spots identified (Marinetti, 1962). Phospholipid spots were cut out from the chromatograms, their radioactivity determined and eluted with 1 N methanolic HCl. The eluates were combined separately, evaporated to a known volume and analysed for phosphorus.

Total phospholipid levels were not affected in liver, kidney and intestine in hypervitaminotic A rats as compared to the control group. The specific activities of liver lysolecithin, phosphatidyl serine, phosphatidyl ethanolamine, and polyglycerophosphatide increased and of monophosphoinositide and lecithin decreased in hypervitaminotic A rats. The levels of lecithin increased and of sphingomylin and lysophosphatidyl ethanolamine and phosphatidyl serine decreased in hypervitaminotic

A rats. The amount of intestine sphingomylin and lysophosphatidyl ethanolamine increased but its specific activities decreased in hypervitaminotic A rats. Kidney phospholipids were not affected.

Technical assistance of Mr N. Srivastava is acknowledged.

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The effects of the level of dietary manganese on shell formation in pullets.

By J. W. MATHERS and R. HILL, *Royal Veterinary College, University of London*

In three experiments involving hybrid pullets, diets of low or high manganese content were given for about 4 weeks before and 4 months after the point of lay, and the effects on shell formation were determined. The low-Mn diet contained 6–7 μg Mn/g and was composed of commonly used feed stuffs. Manganese carbonate to provide 100 μg Mn/g was added to give the high-Mn diet.

There was no difference with level of Mn in the age at which laying began, but among the first few eggs laid by each bird, a higher proportion were shell-less from birds given the low-Mn diet than from those given the high-Mn diet. This difference was confined to about the first twenty eggs produced, and the proportion of shell-less eggs from birds given the low-Mn diet was always greater in the first ten than in the second ten. The magnitude of the effect of the level of dietary Mn on the proportion of shell-less eggs laid varied among the three experiments: it was very small in the first and large in the other two.

Shells formed by birds given the low-Mn diet differed in certain respects from those formed by birds given the high-Mn diet particularly in appearance by both reflected and transmitted light. The shells of eggs from birds given the low-Mn diet had rough surfaces and large translucent areas in transmitted light. These shell abnormalities were striking during the first few weeks of egg laying but became gradually less marked and by about 2 months after the start of laying the differences were small and on casual inspection would probably pass unnoticed.

The mean weight per unit area of shells of eggs laid at the start of egg production by birds given the low-Mn diet was generally lower than the corresponding values for birds given the high-Mn diet. In the first two experiments the difference was highly significant and in the third there was no effect. The first six shelled eggs of each bird were used for this determination. In the third experiment the strength of the same six shells from each bird was measured by determining the force required to pierce the shell with a needle at seven points on the long axis of the egg (Tyler & Geake, 1963). Shells from the low-Mn fed birds were weaker on average than those from the high-Mn fed birds but the difference did not reach statistical significance (low-Mn 693 g, high-Mn 725 g; SE of mean $\pm$26).

During the production of eggs without shells or with abnormal shells the level of dietary Mn did not affect the concentration of plasma Ca.

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An abnormal cartilage formation of unknown nutritional aetiology in chicks. By M. C. NESHEIM and R. M. LEACH Jr. (introduced by K. J. CARPENTER), *Department of Poultry Husbandry, Cornell University and US Plant Soil and Nutrition Laboratory, Department of Agriculture, Ithaca, NY*

An abnormal cartilage development, that occurred when chicks were fed from day-old a purified diet but not when fed a diet composed of normal practical feed ingredients, has been studied in a series of experiments. The purified diet was composed of isolated soya-bean protein (ADM C-1), glucose, maize oil, cellulose, vitamins, minerals, methionine and glycine. The abnormality occurred in the proximal ends of the tibiotarsus and tarsometatarsus of chicks and consisted of a mass of opaque cartilage, irregular in shape and size, below the epiphysial plate extending into the metaphysis. Microscopically the opaque cartilage was composed of a mass of immature chondrocytes with compact, dark-staining nuclei.

The effectiveness of a commercial chick starting ration in preventing the condition is shown by the data in the following table.

Purified diet (%)	Practical diet (%)	Incidence of abnormal cartilage at 4 weeks of age (%)
100	0	60 (20)
75	25	33 (21)
50	50	14 (21)
0	100	0 (20)

No. of chicks per treatment shown in parentheses.

The purified diet was substituted in total by the proportion of practical diet shown in the table.

The purified diet has been tested for adequacy of all vitamins and minerals that are known to affect bone formation, but none of these nutrients have affected the incidence of the abnormal cartilage development. Of the ingredients normally found in commercial chick starting rations, only maize and soya-bean meal showed appreciable activity in preventing the abnormal cartilage development.

The data suggest that a nutritional factor, affecting bone formation is present in natural feed ingredients but not in the purified diet. However, the condition could be the result of an unexplained interrelationship between known nutrients or factors affecting utilization of known nutrients. More data are needed to establish the nature of the dietary effect on cartilage development.

The effect of heat treatment on the limiting amino acids of groundnut flour for the chick. By K. ANANTHARAMAN and K. J. CARPENTER, *School of Agriculture, University of Cambridge*

Grau (1946) found that an extracted groundnut meal as sole protein (20%) source for chicks was lacking primarily in methionine (M) and secondarily in lysine (L). Milner & Carpenter (1963), using both an extracted meal and an expeller flour at 14% protein level, found a large response with M alone, further improvement with M and L and an additional response to a further supplement of threonine (Th). Fisher (1964) using an expeller meal at 12% protein level, however, found that the limiting amino acids were L, Th and M in that order.

Experiments repeating the conditions of Milner & Carpenter (1963) were carried out using de-skinned, milled Spanish groundnuts which were cold-extracted with petroleum spirit after one of the heat treatments shown in the table.

Sample no:	X.513	X.514	X.515
Heat treatment:	Nil	Mild (107°; 0·5 h)	Severe (121°; 4 h)
Analytical data (g/16 g N)			
FDNB-available lysine (Carpenter, 1960)	3·27	3·25	2·04
Streptococcus zymogenes available methionine (Ford, 1962)	1·18	1·23	1·12
Chick response over 10 days			
(Amino acid supplement)		(g gain/g feed eaten)*	
No supplement	0·214	0·197	0·015 (0·046)
0·5% L-lysine (L)		0·168	0·104 (0·137)
0·18% L-methionine (M)+0·14% L-cystine (C)	0·315	0·274	0·030
L+0·24% L-Threonine (Th)	0·213	0·178	0·092 (0·152)
L+C			(0·215)
L+C+Th	0·209	0·191	0·183 (0·214)
L+C+M	0·401 (0·375)	(0·385)	(0·365)
L+C+M+Th	0·540 (0·538)	0·531 (0·528)	0·502 (0·498)

*Figures in parentheses were obtained in a second experiment.

The 'mild' heat treatment apparently had little deleterious effect; the order of limitation in both control (X.513) and 'mild' heated (X.514) flour was again M, L, Th. However, the severely heated X.515 supported poorer growth and showed greatest damage to lysine, the limiting order being L, C, M, Th. Differential damage to lysine has also been found with rats (McOsker, 1962) and by study of enzymic release of amino acids (Mauron & Bujard, 1960). Possibly the meal used by Fisher (1964) was heat-damaged; with other samples (H. Fisher, private communication) there has not been a significant response to lysine alone.

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The growth of young turkeys on diets containing oxidized fish oil. By J. L. L'ESTRANGE and K. J. CARPENTER, *School of Agriculture, University of Cambridge*, and C. H. LEA and L. J. PARR, *Low Temperature Research Station, Cambridge*

As a further test of the usefulness of peroxide values (pv) of the extractable lipids of feeding-stuffs as an indicator of their quality (cf. Lea, Parr, L'Estrange & Carpenter, 1964), turkey hens (Broad Breasted White) were given four treatments from hatching to 6 weeks of age, with three groups of seven poults each per treatment.

Each diet contained 1·5% added fat (either anchovy oil or beef tallow), 10% white-fish meal (contributing 0·7% $CHCl_3$/MeOH—extractable lipid) and 88·5% of a standard 'basal mix' (contributing 3·3% lipid) to give a practical-type diet. The diets included, per kg, 7600 i.u. vitamin A and 12·7 i.u. vitamin E (as α-tocopheryl acetate), both in stabilized form, but no synthetic antioxidant.

The fat was first premixed with the white-fish meal (to simulate an oily fish meal) and then either held at −20° in N_2 or allowed to oxidize as set out below before the preparation of the final diets, which were again allowed access to air for a further storage period in some instances.

	'Fat + white-fish meal' premix				Response of turkeys		
Treatment	Fat used	Period for oxidation at 15°	pv when mixed in diet (μmoles/g)	Storage time (15° in air) between mixing diet and feeding	Live weight gain (g/bird)	Feed conversion ratio	Vitamin A (i.u./liver)
1	Anchovy	Nil	13	3–4½ months	868	1·98 : 1	800
2	Anchovy	3–4½ months	100–60*	Nil	838	1·99 : 1	554
3	Anchovy	3 months	110	3–4½ months	836	2·03 : 1	672
4	Tallow	Nil	2	Nil	890	1·97 : 1	1050
	(Standard errors of treatment means)				(±24)	(±0·028)	(±60)

*This diet was mixed daily and the pv of the premix was falling.

The stored anchovy oil–fish meal premix gave a peak pv of 260 after 65 days' storage; values then declined and continued to do so after mixing with the basal part of the diet for treatment 3.

The turkeys stored vitamin A in their livers on all treatments (from day-old values of 230 i.u./liver), but the quantities stored on treatments 2 and 3 were significantly lower. However the birds showed good appetite and growth rates that were not significantly different even on treatment 3, where the peroxides included when the diet was mixed were some four to five times the conventional limits and where the diets were stored for a considerably longer period than would be considered safe in the feed compounding industry.

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Absence of long-term adaptation in the energy metabolism of sheep on constant feed. By J. L. CLAPPERTON and K. L. BLAXTER, *Hannah Dairy Research Institute, Ayr*

Sixteen wether sheep, eight of the Romney Marsh and eight of the Hampshire

breed aged $2\frac{1}{2}$ years, were given 400, 700, 1000 or 1300 g dried grass daily for 5 months in an experiment which is continuing. At the end of 5 months those given the largest allowance of food weighed nearly twice as much as those given the smallest. Heat production, CH_4 production and faecal and urinary losses of energy were determined with eight of these sheep at 28-day intervals to find whether during continued under- or over-nutrition any adaptation occurred in their energy metabolism. Faecal and urinary losses of energy and the loss of energy as methane were the same in the 5th month as in the 1st month, there being no indication of any change in the ability to ferment and digest any of the four constant rations. The results for body-weight and heat production of eight of the sheep at the end of the 1st and 5th months on constant feed are given in the table. Differences in the weight of the sheep at the end of 1 month largely reflect differences in digestive fill. The sheep given 400 g food lost weight, their metabolism fell very slightly and metabolism/kg $W^{0.73}$ increased slightly. The sheep given 1300 g food increased in weight by 25% and their metabolism increased by 7·4% in the interval. Metabolism/kg $W^{0.73}$ fell slightly.

Table 1. *Means for two sheep of body-weight and heat production when given constant food for 5 months*

Daily allowance of food (g)	Body-weight at end of		Heat production at end of		Heat production /kg metabolic size	
	1st month	5th month	1st month	5th month	1st month	5th month
	(kg)		(kcal/24 h)		(kcal/kg $W^{0.73}$ 24 h)	
400	41·2	38·0	1032	1013	69·1	71·4
700	48·6	51·4	1494	1586	86·3	90·1
1000	51·7	58·3	1819	1919	101·7	98·9
1300	57·1	71·1	2220	2384	115·4	108·5

These results show that once a sheep has adjusted its gut contents and its energy metabolism to a new amount of feed, a process which at maximum takes 2–3 weeks, no further adjustment of metabolism occurs in the ensuing 5 months other than that due to change in the metabolic body size. Subject to the assumption that, irrespective of weight or food intake, basal metabolism is constant/kg weight raised to a power, it can be shown that when the food intake of any animal is increased body-weight increases exponentially, the exponential coefficient being greater the smaller the animal. With sheep the half-time of the exponential is over 400 days. The results of this experiment support these theoretical considerations.

The absorption of vitamin C in the stomach of small animals. By R. E. HUGHES and SANDRA C. LEWIS, *Welsh College of Advanced Technology, Cardiff*

Experiments were carried out to locate the main site of absorption of vitimin C in the gastro-intestinal tract of the guinea-pig, rat and hamster, and to examine the effect of factors likely to influence the absorption.

Four ml of a solution of ascorbic acid (10 mg/100 g body-weight) in water, 0·1N-HCl or 0·25 M-phosphate buffer according to the nature of the experiment, were injected into the stomach or intestine of the anaesthetized animal. Blood

samples were removed at 3 min intervals by cardiac puncture through the diaphragm and the ascorbic acid content determined.

In the three species examined there was rapid absorption of the vitamin from the stomach, the blood level reaching a maximum value within 10 min of the time of injection. Absorption from the intestine was slower, more prolonged and in general conformed to the usual pattern for intestinal absorption (Wisemann, 1964). The pH of the injection medium (0·1 N-HCl, 0·25 M-phosphate buffer pH 7·4 etc.) appeared to have little effect on the course of absorption.

In vitro incubation experiments both with the intact stomach and with portions of stomach tissue also demonstrated an uptake of ascorbic acid. Homocysteine (but not homoserine) depressed the uptake of ascorbic acid by stomach tissue at pH 7·4. At this pH there would be spontaneous conversion of ascorbic acid (vitamin C) to dehydroascorbic acid (DHAA), a reaction that is reversed by homocysteine (Hughes, 1956). It would therefore appear that in the absence of free acid vitamin C is at least in part absorbed as DHAA—a not unexpected finding in view of the recent demonstration that at pH 7·4 DHAA crosses the erythrocyte membrane some five times as quickly as ascorbic acid (Hughes and Lewis, unpublished work).

It is concluded that in the three species examined the bulk of the ingested vitamin C is absorbed in the stomach and that the absorption is not dependent upon the acidity of the stomach contents.

Medical Research Council support for this project is gratefully acknowledged.

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Refractive error in the rat. By P. G. WARD, *Nutritional Ophthalmology Research Unit, East African Institute for Medical Research, Mwanza, Tanzania*

The progeny of rats, already on diets 'N' and 'B', were weaned onto their own dam's diet and remained thereon until death. The diets were a commercial mouse meal ('N'; NDpCals=10·5%), and this feed diluted with an equal part, by weight, of cornflour ('B'; NDpCals=6·4%). Fat-soluble vitamins were given orally to the 'B' rats to ensure, among other things, that they received adequate vitamin A. Gardiner & Macdonald (1957) have shown that dietary protein appears to be a factor in rabbit myopia, whilst Johnstone & McLaren (1963) considered dietary vitamin A, rather than protein, might have the greater importance in human myopia.

Refractions (218 and 316) were made on twenty-seven 'N', and fifty-three 'B' rats respectively. All the rats were over 30 days old when first 'sight-tested', under cycloplegic (0·1% aqueous hyoscine hydrobromide), with a standard set of trial lenses and a retinoscope.

At killing, the eyes of forty-seven 'N', and fifty-six 'B' rats were enucleated for globe and lens fresh weight, and lens dry weight determinations: the latter were measured after 48 h at 100–110°.

Refractive errors, fresh globe, and fresh and dry lens weights were found to be independent of sex.

With ageing, the normal rat refractive error progressed from high myopia to high hypermetropia, but the trend was inhibited by the lower NDpCals% of diet 'B' (Fig. 1).

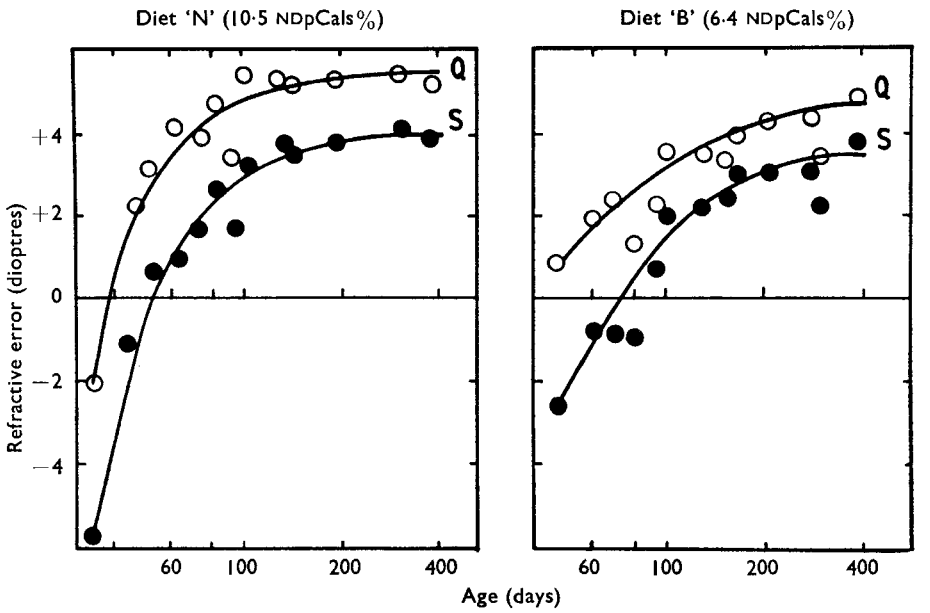

Fig. 1. Changes in rat refractive error with ageing for diets 'N' and 'B'. Spherical (S) and equivalent spherical (Q) errors.

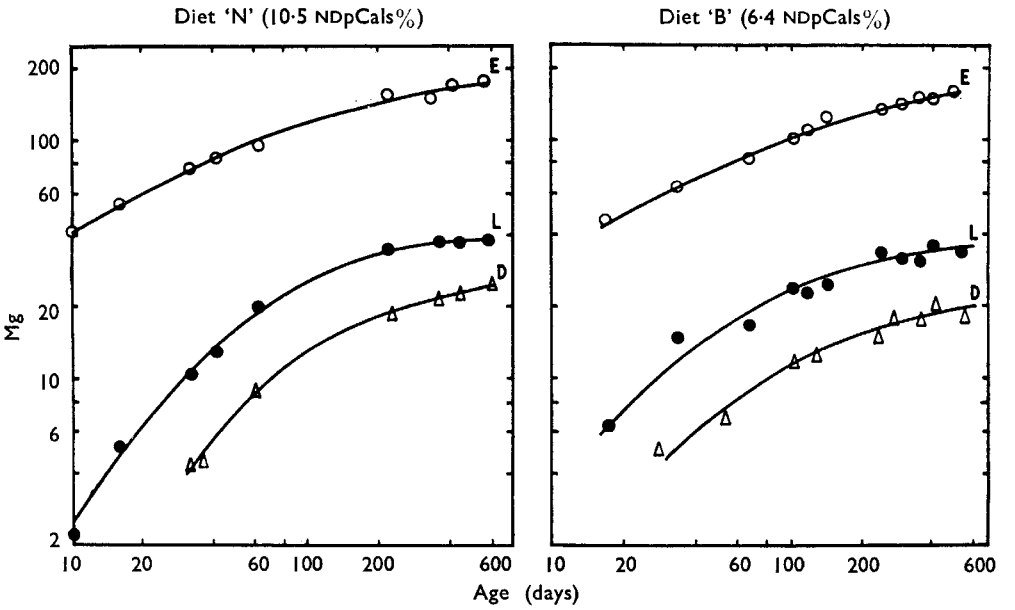

Fig. 2. Changes in rat fresh eyeball (E), fresh lens (L) and dried lens (D) weights with ageing for the two diets 'N' and 'B'.

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Throughout life, there was a steady increase in fresh globe, and fresh and dry lens weights with those of the diet 'B' rats consistently lighter than those from the normal rats (Fig. 2).

Therefore in the rat, reduced dietary protein, in the presence of adequate dietary vitamin A, induced more myopia, or less hypermetropia depending on age, through the retardation of globe and lens growth.

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Urinary sulphate sulphur as a measure of the protein value of diets.

By P. L. PELLETT, *London School of Hygiene and Tropical Medicine, Keppel Street, London, WC*1

In the recent report of the (USA) National Research Council (1963) it was suggested that urinary sulphate sulphur/nitrogen ratios were a possible index of the quality of protein consumed and hence sulphate sulphur/creatinine ratios may indicate overall protein value of a diet i.e. quality and quantity. Miller & Mumford (1964) have shown that the total inorganic sulphate excretion per 24 h is proportional to net dietary protein consumed and Lassalle, Taggle & Donoso (1964) have demonstrated a linear relationship between the urinary S/N ratio and that of the diet. The present results are a continuation of the work reported to the NRC conference by the author.

Eight diets were fed to groups of students (five or eight subjects per group) at the Massachusetts Institute of Technology for 4-day periods, 24 h urine samples being collected for the latter 2 days, these were analysed for total nitrogen, creatinine, inorganic sulphate sulphur and total sulphate sulphur. A rapid turbidimetric procedure was used for inorganic sulphate sulphur similar to that of Berglund & Sörbo (1960), using a reagent containing 1% $BaCl_2$ and 0·3% gelatin in 0·1 N-HCl. This gave an extremely high correlation ($r=0{\cdot}998$) with the standard gravimetric procedure.

The diets fed were based on cereals, legumes or egg and the range of NDpCal% was from 4·4 to 9·9 as determined by rat feeding tests. The mean excretion of inorganic sulphate sulphur per 24 h ranged from 324 mg to 883 mg.

The calorie intake of all subjects on all diets was similar thus it was possible to compare urinary ratios with the composition of the diet. The following comparisons all gave high correlation coefficients ($r=0{\cdot}92-0{\cdot}94$); NDpCal% versus 24 h sulphate excretion, NDpCal% versus sulphate excretion per g creatinine, sulphur amino acid score versus urinary sulphate to nitrogen ratio. These comparisons were for both inorganic sulphate sulphur and total sulphate sulphur. Calculations showed that $11{\cdot}6\pm0{\cdot}60$ mg inorganic sulphate sulphur and $13{\cdot}0\pm0{\cdot}54$ mg total sulphate sulphur

were excreted per g net dietary protein consumed. The value for inorganic sulphate sulphur was extremely close to that reported by Miller & Mumford (1964).

Since postprandial excretion curves for sulphate and nitrogen do not parallel each other, 24 h samples would appear to be necessary. However, further work is necessary to show whether shorter periods of collection, say 5–8 h, may in fact be adequate.

This work was completed whilst the author was in the Department of Nutrition and Food Science, Massachusetts Institute of Technology.

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French moult—a disease of the budgerigar of nutritional origin. By T. G. TAYLOR, PHILIPPA M. TINDAL and C. BISHOP, *Department of Physiological Chemistry, University of Reading*

Nestling budgerigars are prone to suffer from a disease known as French moult, characterized by the shedding or breaking off of the flight and tail feathers, usually between 28 and 40 days of age and by a reduction in the haematocrit value of the blood. The normal diet of these birds consists of canary seed and various types of millet, together with cuttlefish bone and grit. The evidence suggests that French moult is caused by a nutritional deficiency. However, we have supplemented a normal seed diet with all nutrients known to be dietary essentials for avian species without reducing the incidence of the disease.

We have shown recently that a hypervitaminosis A in Japanese quail (*Coturnix*) exhibits a number of features in common with French moult in budgerigars and the possibility that the latter disease is caused by a hypervitaminosis A has been considered, since many breeders feed cod-liver oil to breeding birds by adding it to the seed at a level of 1%. The results of experiments in which budgerigar chicks were fed vitamins A and D will be reported.

DEMONSTRATIONS

The use of the mouse for the study of iron in nutrition. By P. V. J. HEGARTY and G. R. WADSWORTH, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1*

It is sometimes important to know the total quantity of iron in the body. This is impossible to measure directly in man and large experimental animals. The validity of estimating total body Fe from the amount in aliquots of the whole carcass

or from a single tissue such as liver is often open to question. This is because of the difficulty of selecting representative samples and also because the disposition of Fe in different parts of the body varies according to the stage of development of the animal (Widdowson & McCance, 1951).

The mouse has several advantages as an experimental animal for the study of Fe in nutrition. The quantity of Fe in the whole carcass can be determined; it is feasible to measure blood volume and from this estimate the proportion of Fe in circulation and in the tissues; the life span is short enough to allow study through the various developmental and physiological stages of life (Hegarty & Wadsworth, 1963*a*).

Using a technique (Hegarty & Wadsworth, 1963*b*) based on Wootton's (1958) method, the amount of carcass Fe in the mouse is being studied in relation to the diet. One objective is to define conditions for a standard biological test for availability of Fe from various foods and diets.

Some preliminary results using wheat bread, rice and a standard laboratory diet fed to weanling mice are shown in the table.

Table 1. *Amount of iron in the carcasses of mice fed on different diets*

Diet	Weeks on diet	No. mice	Mean wt. (g)	Total iron per mouse (μg)
Start of experiment		6	8·9	588
41B	4	10	27·1	1391
Rice	4	10	28·0	1106
41B	7	10	30·4	1661
Rice	7	10	30·9	1287
Start of experiment			10·2	723
41B	4	5	26·0	1489
Bread	4	4	25·0	1081

The test diets consisted of bread, and long-grained brown rice supplemented in each instance with a vitamin mixture, an Fe-free salt mixture and vitamin-free casein and had an NDpCal% of 11·8 for the bread diet and 11·3 for the rice diet. This supplementation was necessary in order to sustain growth. The Fe content, in mg Fe/100 g dry diet, of 41 B, rice and bread diets was, respectively, 12·0, 2·9 and 3·7.

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Comparison of group tests for the assessment of diet and nutritional state.

By BERNARD T. SQUIRES, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW7* and T. P. EDDY and HELMY RIAD GAYED, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1*

Squires (1958, 1961, 1963) described changes in the buccal mucosa of African patients suffering from protein-calorie deficiency, and by animal experiments, demonstrated a correlation between these changes and the protein value of the diets. Changes in staining properties of the cells are attributable to keratinization and may be demonstrated by the Falg stain of MacConnail & Gurr (Gurr, 1962). Non-cornified cells stain blue-violet, keratinized cells scarlet. Buccal scrapings taken from adolescents in three institutions for handicapped or deprived children near London have been compared with scrapings from normal adolescents at two public schools. Mean values and standard errors for the percentage of non-cornified cells were: seventy-six normal public schoolboys $78{\cdot}2 \pm 1{\cdot}2\%$; fifty-nine adolescents at a hospital for the mentally subnormal $69{\cdot}2 \pm 2{\cdot}0\%$; fifty-one adolescents at a school for spastics $42{\cdot}3 \pm 1{\cdot}7\%$; seventy-one orphans and homeless at an orphanage and hostel $46{\cdot}1 \pm 1{\cdot}1\%$.

The percentage of urea nitrogen to total N in a single specimen of morning urine has been taken to indicate the amount of protein in the diet (Platt, 1954, 1958; Luyken & Luyken-Koning, 1960). Mean values with standard errors were: seventy-six public schoolboys $85{\cdot}3 \pm 0{\cdot}8\%$; forty-one mentally subnormal $78{\cdot}5 \pm 1{\cdot}5\%$; seventy-five spastic adolescents $76{\cdot}0 \pm 1{\cdot}0\%$. The differences are similar to those found with buccal smears.

Urea N : total N (%)	% non-cornified cells in buccal smear						Total no. of subjects
	Under 50	50–	60–	70–	80–	90+	
90+	3, 8·8%	5, 14·7%	7, 20·6%	11, 32·4%	7, 20·6%	1, 2·9%	34
80–	14, 16·7%	18, 21·4%	8, 9·5%	23, 27·4%	18, 21·4%	3, 3·6%	84
70–	25, 37·8%	17, 25·8%	9, 13·6%	5, 7·6%	7, 10·6%	3, 4·5%	66
60–	9, 37·5%	6, 25·0%	1, 4·2%	6, 25·0%	1, 4·2%	1, 4·2%	24
50–	0, 0%	4, 50·0%	3, 37·5%	1, 12·5%	0, 0%	0, 0%	8
Under 50	3, 33·3%	2, 22·2%	3, 33·3%	1, 11·1%	0, 0%	0, 0%	9
Total	54, 24·00%	52, 23·11%	31, 13·78%	47, 20·89%	33, 14·67%	8, 3·56%	225

Analysis of variance: $\chi^2 = 47{\cdot}65$ $n = 25$ $P < 0{\cdot}005$.

The table demonstrates a relationship, in 225 subjects who received both tests, between urea N : total N—a measure of ingested protein—and the degree of keratinization of buccal cells—a measure of the clinical effect of minor differences in nutrition.

Dietary measurements gave values of NDpCal% 7·0–7·5 for school food taken by spastic adolescents and NDpCal% 8·0 for hospital food taken by the mentally subnormal. An estimate for the normal controls is NDpCal% 8·5.

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Demonstration of computer programmes applicable to nutritional calculations. By P. L. PELLETT and ERICA F. WHEELER, *Department of Human Nutrition, London School of Hygiene and Tropical Medicine, London WC1*

The calculations involved in nutrition and dietetics are simple, though tediously repetitive, and comparatively short computer programmes can be written to deal with them. Two such programmes will be demonstrated.

Optimal. The protein value of a diet depends on the mutual supplementary effect of the proteins of different foods. It is possible to calculate the protein value of all possible combinations of a given number of food items and decide which combination gives the highest protein value for the lowest cost. The present programme calculates the protein-calorie value of mixtures containing up to ten food items in all possible proportions, increasing each food item present by any desired amount (provided that each increment is a factor of 100). In order to avoid unnecessary calculations only those combinations are processed whose available protein content is adequate, and whose total cost is less than twice that of the staple food concerned. The compositions of the mixtures which meet these requirements are printed, together with protein calories %, cost per 100 g of mixture, and protein scores on the basis of sulphur amino acids, lysine and tryptophan. The lowest score is used for calculating the NDpCal% and net dietary protein (in g). A final column gives the quantity of net dietary protein per unit cost, which enables mixtures giving maximum protein value for minimum cost to be selected.

Nutrient evaluation. In this programme the composition of a diet in terms of twenty nutrients (protein, calories, fat, minerals and vitamins) is calculated from the weights of foods eaten and number of subjects surveyed. A simple mnemonic coding system is used to identify foods and meals. The diet may be evaluated in terms either of meals or of total 24 h intakes. Derived data, such as protein-calories % and NDpCal%, are also calculated. Much of the time spent in developing such a programme is given to the collection of food composition data, especially when a wide range of composite dishes is being used. Survey results can now be computed rapidly.

Further programmes of a conventional statistical nature will be demonstrated, which have been adapted for dealing with biochemical and clinical survey data.

All the programmes demonstrated were written either in Autocode or in Fortran for use on the London University Atlas computer.

Changes in the alimentary canal produced by protein–calorie deficiency. By B. S. PLATT and R. J. C. STEWART, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW7*

Morphological changes in the alimentary canal have been described in infections, infestations and other conditions such as idiopathic steatorrhoea. The relationship of these conditions and the part played by intestinal pathogens in the development of clinical symptoms such as diarrhoea are of considerable interest.

The role of protein-calorie deficiency in the development of the morphological abnormalities is often ignored. The demonstration will include changes in the alimentary canal produced in animals maintained on protein-calorie deficient diets.

The effect of the protein value of the diet on the body composition of the rat during pregnancy. By DONALD J. NAISMITH, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW7*

Estimation of the mineral density of the human phalanx from radiographs. By P. R. PAYNE and R. J. C. STEWART, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW7*

Two approaches have been made in the past: (1) the selection, as representative of the whole, of defined areas of the radiograph (Kean, Spiegler & Davis, 1959), (2) the use of a scanning microdensitometer and integration of the curves obtained (Mack, O'Brien, Smith & Bauman, 1939). Both methods avoid the large range of optical densities (OD) resulting from the irregular shapes of the bones and the uneven distribution of mineral. However, the selection of suitable areas for the first method is based on arbitrary criteria and the second needs expensive equipment and lengthy analysis of data.

The method demonstrated relies on the fact that only a small range of OD (0·2 units) occurs in a narrow, central, longitudinal strip of the radiograph of a phalanx. The radiographs taken on Ilfex film, tube to film distance 36 in. are developed in Phen-X for 5 min at 20°. The complication due to soft tissue is eliminated by immersing the hand and an aluminium step-wedge under 5 cm of water. Exposure is for 0·5 sec at 48 kV 22 mA.

The densitometer has an aperture 3 mm wide whose length can be adjusted to that of the bone. Single readings are obtained from radiographs of the bones and each step of the wedge. The average bone mineral/cm^2 is calculated from the equivalent aluminium (Al) thickness, using the factor: 1 mm Al$\equiv$130 mg bone mineral/cm^2 (Keane *et al.* 1959). To determine the ash/cm^3 the average thickness of the bone has been estimated by determination of average width and application of the ratio (width : thickness :: 1·57 : 1) derived from direct measurement of thirty proximal phalanges of middle fingers. The technique has been applied to thirty-three autopsy specimens whose ash contents and volumes have been directly measured (r=0·59). Predictions using lateral radiographs to obtain average bone depth are as good as, or better than, those using the above factor.

The method offers sufficient accuracy to follow changes in the density of bone during longitudinal studies of adult subjects.

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The effect of the protein value and calcium content of the diet on the long bones of rats. By N. R. H. EL-MARAGHI, *Human Nutrition Research Unit, National Institute for Medical Research, Mill Hill, London, NW7*

Osteoporosis develops in the bones of young growing animals maintained on diets of low protein value or containing inadequate calcium. The most marked changes occur when the diet is low in calcium and has a high protein value. On a diet of low protein value there is a reduction in the thickness of the epiphysial cartilage, the length of the bony trabeculae, osteoblastosis and the thickness of the wall of the shaft. In the bones of animals given diets of inadequate calcium content there is increased osteoclasis and thinning of the cortical wall without a reduction in osteoblastosis.

In adult rats given diets of low protein value, osteoblastosis is reduced early and more markedly than is osteoclasis; this relatively excessive osteoclasis leads to thinning of the wall of the shaft. In adult rats the epiphysial cartilage is inactive; there is therefore no alteration in the length of the bone. In this age group the lowest concentration of calcium fed (0·11%) appears to be sufficient to protect against mineral-osteoporosis and only small increases in ash content occurred when the concentration of calcium in the diet was quadrupled.

Reproduction imposes an increased requirement for nutrients in the mother, which, if not supplied, leads to alterations in her bones. The effects of gestation and lactation are very different. At the end of gestation the bones appeared to be little different from those of non-pregnant animals of the same age which had been maintained on similar diets. During lactation, however, the bones of all the dams that reared litters, even those given the high-protein diet with 0·44% calcium, showed some degree of rarefaction. The rarefaction was more marked in animals given diets with lower protein or calcium concentrations and it was the dams given the high-protein, low-calcium ration whose bones developed severe osteoporosis.

Thus, in young growing rats and nursing dams, both of which groups have a high requirement for protein and calcium, severe mineral-osteoporosis occurs when the protein, but not the calcium, needs are satisfied. In other groups in which the demand for protein and calcium are lower, matrix-osteoporosis predominates.