Digestion in the crop of the fowl. By W. BOLTON, *Agricultural Research Council Poultry Research Centre, West Mains Road, Edinburgh, 9*

Food in the crop may be acted upon by the bacterial flora, and by the enzymes secreted by the fowl and in the food.

Preliminary experiments had suggested that hydrolysis of starch to sugar, and the production of lactic acid, were the major changes taking place in the crop. In a study of these phenomena four adult male fowls were allowed access to food for 1 h, and then killed, 1, $2\frac{1}{2}$, $4\frac{1}{4}$ or 6 h after feeding, and the crop contents were analysed. The results, set out in Table 1, show that sugar content increased during the first $2\frac{1}{2}$ h, with little change in the other components studied. Thereafter the

Table 1. *Contents of the crop at various periods after feeding (dry-matter basis)*

		Time after feeding (h)			
	Mash	1	$2\frac{1}{2}$	$4\frac{1}{4}$	6
pH	5·9	6·1	6·1	5·2	4·5
Sugar (%)	1·3	4·0	3·7	3·3	1·6
Ethyl alcohol (mg/100 g)	9	13	6	18	109
Acetic acid (%)	0·01	0·02	0·01	0·10	0·13
Lactic acid (%)	0·2	0·2	0·2	1·7	3·2

sugar content and the pH fell, and the contents of alcohol, acetic and lactic acids increased. This is suggestive of a period wherein amylase activity predominates followed by a period when the multiplication of lactobacilli is the most important feature.

The One Hundred and Fiftieth Meeting of The Nutrition Society was held at the Zoological Society of London, Regent's Park, London, N.W.1, on Friday, 25 May 1962, when the following papers were read:

Arginine metabolism and nutrition in the chick. By G. H. SMITH and D. LEWIS, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough*

Arginine is an essential amino acid for the chick but the level required in the diet seems to vary with the overall amino acid composition of the diet. The apparent requirement ranges from 2·3% on diets mainly based on casein to around 0·9% on diets of a more usual commercial type. An examination of this problem has been made in terms of the metabolic interrelationships of arginine. Arginine has two major functions in the body; as a constituent of protein and as a donor of amidine groups for creatine synthesis. It is also attacked by arginase to yield urea and ornithine: as the urea cycle is not operative in avian species this may represent a wasteful pathway.

The characteristics of chick arginase have been studied and found to be similar to those of the mammalian enzyme; optimum pH 9·5 and K_m of $6{\cdot}7 \times 10^{-3}$ M. Arginase is active in the kidney of the chick and also to some extent in the liver. Arginase activity in the rat and chick has been compared in the table.

Arginase activity expressed in μmoles urea produced per h per g wet weight of tissue

	No. of animals	Activity	SD
Rat liver	14	117 000	12 000
Rat kidney	14	2 250	500
Chick liver	15	380	190
Chick kidney	15	4 000	2 200

The enzyme glycine transamidinase occurs both in the kidney and the liver of the young chick. It is, however, considerably less active than arginase.

A study has also been made of arginine requirement using rations of the type encountered in the United Kingdom. Rations were prepared based upon wheat, maize, soya-bean meal, groundnut meal and maize-gluten meal. Two series of trials were carried out. In one case protein and arginine levels were progressively lowered and in the other protein level was maintained at 20% whilst the arginine level was reduced. In no case did arginine supplementation improve growth or food conversion and it is evident that at a protein level of 20%, 0·7% arginine is adequate. It was established that growth was not limited by another factor since lysine addition resulted in reduced growth, which could be reversed by arginine supplementation.

Digestibility of fats and fatty acids in the pig. By H. S. Bayley and D. Lewis, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough*

The lack of knowledge on the digestion of fat by herbivores, and the commercial possibilities of fat inclusion in the rations of farm animals, has prompted an investigation of the digestibility of fats in the pig.

Three experimental rations were used: a basal ration with no added fat, a ration containing 5% of a hydrolysed fat mixture (50% free fatty acids) and a ration containing 5% of beef tallow. The rations based on standard ingredients had similar protein levels and each was fed to four male Large White pigs (20 kg). During a period in a metabolism cage faeces and urine were collected separately.

Samples of food and faeces were extracted with light petroleum (40°–60°) in a Soxhlet apparatus; a further lipid extract was obtained by soaking the residues in a 10% (v/v) solution of glacial acetic acid in light petroleum followed by a Soxhlet extraction (Carroll & Richards, 1958). Calcium soaps were present to some extent in the food and to a considerable extent in the faeces. The extracts were saponified: the free acids were refluxed with acidified methanol and the methyl esters analysed by gas–liquid chromatography.

The fatty acid balance for the pigs fed the basal ration showed that unsaturated acids had been largely replaced by saturated acids. The faecal fat on this ration,

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therefore, could not be used to indicate endogenous fat production. Values for correcting the faecal fat have thus been obtained by feeding a diet prepared by extracting the basal ration in a large Soxhlet apparatus.

The results obtained in these studies indicate that beef tallow has a slightly higher corrected digestibility than the hydrolysed fat mixture (HEF no. 1). On the whole the unsaturated fatty acids were better utilized than the saturated ones, the digestibility of stearic acid being particularly low.

Two further experiments were carried out in which oleic and stearic acids were fed both as free acids and as triglycerides. There was no indication that dietary oleic acid was saturated in the alimentary tract and the acids were utilized to comparable extents whether free or esterified (Table 1).

Table 1. *Corrected digestibility of fats and fatty acids in the pig*

	No added fat	5% HEF* no. 1	5% beef tallow	5% triolein	5% oleic acid	5% tri-stearin	5% stearic acid
Total lipid	87	76	86	96	97	55	57
Myristic acid	83	88	98				
Palmitoleic acid	100	100	100				
Palmitic acid	93	64	73				
Oleic acid	92	96	97	98	98		
Stearic acid	0	0	47			52	44

The endogenous fat output was 11·5 g lipid/day, containing 0·17 g myristic, 0·10 g palmitoleic, 2·28 g palmitic, 0·95 g oleic and 4·66 g stearic acids.

*Thomas Hedley Ltd, Newcastle upon Tyne.

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Digestible energy value of cereals to pigs. By D. W. ROBINSON and D. LEWIS, *University of Nottingham School of Agriculture, Sutton Bonington, Loughborough*

There are several methods that may be used to define the 'energy' value of pig foods, most of which attempt to improve upon the traditional use of the term total digestible nutrients (TDN). Productive or net energy values are extremely difficult to determine accurately using the pig. Digestible or metabolizable energy values on the other hand are relatively simple to assess and give a more consistent indication of the characteristics of the diet. The term digestible energy is recommended since it gives a representation of that which is potentially available irrespective of the metabolic status of the animal.

The determination of digestible energy of cereals was carried out using sixteen male Large White pigs, four/treatment. During the 'finishing' period from 120 to 200 lb live weight the pigs were fed rations that contained 97·5% yellow maize, wheat, barley or milo respectively for the four treatments. The pigs were maintained on two separate occasions in metabolism cages and following 7 days acclimatization,

the faeces and urine were collected for 5 days. By standard procedures of analysis the digestible energy values were obtained (Table 1).

Table 1. *Digestible energy values (kcal/g) of cereals compared with those recorded by Diggs, Becker, Terrill & Jensen* (1959). *The corrections of Hill & Anderson* (1958) *have been applied*

Cereal	Diggs *et al.* (1959)	Present results
Maize	3430	3430
Wheat	3250	3300
Barley	—	2880
Milo	3240	3300
Oats	2830	—

Since it is recognized that the total combustible energy of protein is not potentially available to the animal a correction can be made for the nitrogen that is absorbed. Corrected values (Hill & Anderson, 1958) are also included in Table 1.

Attempts are often made to calculate digestible energy from TDN values, usually by using a factor of approximately 20 on a lb basis. By assuming a factor of 44 on a kg basis it is possible to calculate equivalent TDN values of 78 for maize, 77 for wheat and milo and 66 for barley.

In considering 'energy' in relation to feeding-stuffs it must be emphasized that there is no general satisfactory method of evaluation independent of the rations in which they are incorporated and it is more satisfactory to evaluate a complete ration.

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Vitamin C supplement for suckling pigs. By R. S. Barber, R. Braude and Patricia Cooke, *National Institute for Research in Dairying, Shinfield, Reading*

Russian workers (Gorb & Ros, 1956) reported that suckling pigs had benefited from supplementation of their normal rations with vitamin C. These results were contrary to evidence obtained at this Institute that the baby pig gets all the vitamin C it requires either from its mother's colostrum and milk, which are rich in vitamin C, or by synthesizing its own vitamin C (Braude, Kon & Porter, 1950). A test was therefore carried out in which 162 suckling pigs in twenty litters in our herd received daily oral doses of 75 mg ascorbic acid from the 5th day until they were 5 weeks old (treatment A); twenty control litters with 163 pigs were similarly dosed with distilled water (treatment B). The table below gives mean values for live weight at 5 and 8 weeks of age and creep feed consumption during the 8-week period together with their standard errors (for 16 df). Analyses of covariance were carried out taking into account the variable number of pigs in the litters. There was no significant difference between treatments.

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	Weight at 1 week (lb)	Weight at 5 weeks (lb)	Weight at 8 weeks (lb)	Creep feed consumption (lb)
Treatment A	5·69	19·01	33·51	10·87
Treatment B	5·63	19·03	33·36	11·35
A–B	0·06	−0·02	0·15	0·48
Standard error	±0·3397	±0·7111	±1·4468	±1·3112

We wish to thank Roche Products Ltd, London, for the gift of the ascorbic acid and for supplying it in ampoules containing 75 mg each.

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Corticosteroid excretion in experimental protein–calorie deficiency in pigs. By PAMELA A. J. DURBIN and C. R. C. HEARD, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Protein-deficient pigs have been shown to have impaired glucose tolerance (Heard, Durbin & Platt, 1961). This may be due to reduced insulin supply or excess insulin antagonists, including possibly glucocorticoids. The increased accumulation of glycogen and fat in the livers of these animals (Durbin, Heard & Platt, 1960) and an increased sodium : potassium ratio in their muscles (Platt, 1962) supports this possibility. Therefore the urinary excretion of corticosteroids was investigated.

Pigs were maintained on low-protein (LP), low-protein with additional carbohydrate (LP + CH), low-protein in which 5 or 20 g casein replaced equal weights of starch in each 100 g of diet (5CLP or 20CLP) or normal (Nl) diets (Friend, Heard, Platt, Stewart & Turner, 1961). Collections (24 h) of urine were analysed for free 17-ketosteroids (17-KS), 17-ketogenic steroids (17-KGS) (Norymberski, Stubbs & West, 1953) and total 17-hydroxycorticosteroids (17-OHCS) (Appleby, Gibson, Norymberski & Stubbs, 1955).

Expt no.	Age (days)	Diet	Sex	Weight (kg)	mg excreted/24 h 17–KS	17–KGS	17–OHCS	17–OHCS (μg/kg 24h)
1	85	Nl	♂	32·5	4·6	3·4	7·2	222
		Nl	♀	30·0	1·6	0·7	2·3	76
		20CLP	♀	12·4	1·1	2·5	3·7	301
		LP+CH	♀	9·0	0·4	2·0	2·2	244
2	105	LP+CH	♂	6·1	0·6	1·9	2·0	328
		LP	♀	5·2	0·7	2·7	3·2	625
3	167	20CLP	♀	22·0	0·5	3·8	4·4	201
		LP 20CLP*	♀	17·6	0·6	3·3	3·4	195
		LP	♀	13·5	0·2	2·9	3·2	239
		LP+CH 20CLP*	♀	17·7	0·4	3·0	3·2	181

*Diet change at age 121 days.

As can be seen from the above table the deficient animals maintained a steady steroid excretion which may be less than the output of the normal pig at the same age, but when expressed per kg body-weight, was at least as great as that of the normal pig and, in the most severely malnourished animals (Expt 2), was considerably greater than normal. Similar results have been obtained with rats on non-protein diets.

Therefore in protein-calorie deficiency in which the secretory activity of other endocrine organs appears to be impaired viz. pancreas (Stewart & Heard, 1960) and thyroid (Platt & Stewart, 1962), the adrenal cortex continues to function normally. The result is an endocrine imbalance, a feature of which is a relative excess of adrenal steroids.

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Differences in the blood of dogs due to diets of different protein value.

By B. S. PLATT, C. R. C. HEARD, R. J. C. STEWART and H. A. AL-RABII, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Robertson (1962) has stated that genetic influences alter the response to unfavourable nutrition and conversely that suboptimal nutrition increases genetic variations. The effect of diets of suboptimal protein value on the behaviour of a number of blood constituents has been studied in dogs, using litter-mates as controls. Four diets have been used: (1) U.K.II (ND-p Cals = 8·6%) is equivalent to an average 'United Kingdom' diet; (2) U.K.II.C—U.K.II reduced to ND-p Cals = 6·8%. The effect of this diet on the nervous system of dogs has been described (Platt, 1962). Diets 1 and 2 differ in factors other than protein value; therefore diets 3 (ND-p Cals = 12%) and 4 (ND-p Cals = 6·3%), differing only in their protein values, have also been used.

Eight litters of four dogs each were used. In four litters, two dogs from each litter were fed on diets 1 and 2, and in the other four litters, two dogs from each were fed on diets 3 and 4. For each pair of diets, the dogs on the diet of lower protein value, had reduced serum protein (principally albumin), haemoglobin, RBC, serum vitamin A and glucose tolerance, in agreement with our previous findings of the effects of protein-calorie deficiency in pigs. The increase in serum iron in the animals which were fed on the lower-protein diets may reflect decreased rate of erythropoiesis due to shortage of the protein moiety (globin) as well as to shortage

of transferrin which chelates iron for its transference to the tissues. Moreover the uptake of iron by the tissues would be limited by shortage of apo-ferritin (Mazur, Baez & Shorr, 1955). The unsaturated iron-binding capacity (UIBC) of the serum showed a reciprocal relationship to the serum iron.

Blood of dogs on diets of different ND-*p Cals* %

ND-p Cals % of diet	8·6 (UK.II)	6·8 (UK.IIC)	12·0	6·3
Body-weight (kg)	11·7	9·9	12·1	9·3
Serum total protein (g/100 ml)	6·42	5·35	6·78	5·43
Serum albumin (g/100 ml)	4·30	2·96	4·38	3·10
Serum vitamin A (i.u./100 ml)	358	308	519	326
Hb (g/100 ml)	14·80	11·00	14·89	11·60
RBC(10^{-6}/mm^3)	6·00	5·10	6·20	4·53
Serum iron (g/100 ml)	138	156	126	163
UIBC (g/100 ml)	249	202	240	165
TIBC (g/100 ml)	387	358	366	328
I.V. glucose tolerance (slope of curve 'K')	5·3	3·6	7·7	4·6

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The chick embryo as a test organism for toxic substances in food. By B. S. PLATT, R. J. C. STEWART and S. R. GUPTA, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

There are many substances in foodstuffs, both naturally occurring and 'additive', which have, or are suspected of having, adverse effects on the consumer. The effects may vary from species to species with age and stage of development, as well as with the nutritional state of the subject. Morcos & Platt (1962) have demonstrated the importance of the protein value of the diet in the development of osteolathyrism. Noxious influences have a greater effect the younger the subject and maximal effects can be expected during embryonic development. Tests on mammalian species are laborious and expensive, but the introduction of test substances into the yolk of the hen's egg is simple.

Five-day chick embryos were used. β-Aminopropionitrile fumarate in doses of 400 μg caused death within 6 days and all embryos given 150 μg or more died or developed skeletal lesions, confirming the observations of Chang, Witschi & Ponseti (1955). Methionine sulphoximine in doses of 200 μg caused skeletal deformities (Mellanby, 1956) and larger doses of 400 μg or more led to a high proportion of deaths. The Tropical Products Institute (TPI) invited us to test several preparations of 'groundnut toxin'. Eggs were injected and examined after 2 days; as little as 0·3 μg of the more potent samples caused death. The results correlated well with those obtained on day-old ducklings (communication from TPI), but the quantities

required for a positive result were much smaller (about $\frac{1}{200}$). Growth of chick embryo was reduced for 3–4 days after injection with α-phthalimidoglutarimide (thalidomide), but the effect of the size of the particles—thalidomide is only slightly soluble in physiological solutions—has yet to be determined. Thalidomide has no significant effect when the treated embryos are allowed to hatch, although Somers (1962) has demonstrated an effect on mammalian embryos. Bevelander, Nakahari & Rolle (1959) have shown that the tetracyclines are selectively deposited in the skeleton and that 2·5 mg may inhibit growth.

Suspected substances should not be permitted in foods for human consumption until tests have shown them to be harmless for the most sensitive tissues. Such tests could conveniently be made on the avian embryo.

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The effect of the protein value of the diet on the toxicity of β-amino-propionitrile. By S. R. Morcos and B. S. Platt, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

β-Aminopropionitrile (BAPN) produces osteolathyrism in rats. There is evidence, which is sometimes contradictory, that the effects of the lathyrus toxin are influenced by the amount of protein in the diet (Gardner, 1959; Lalich, 1960).

Diets of different protein values, ND-p Cals% = 10·1, 6·2, 4·6, 4·6 restricted to 75% *ad lib.* intake, 3·7, 2·1 and zero were prepared. Each diet was tested two or three times on three groups of rats of similar body-weight (46–48 g). One group was fed on the diet supplemented with BAPN, one of the two control groups was 'pair-fed' on the toxin-free diet and the other was fed the same diet *ad lib.* The amount of BAPN (as fumarate) mixed with each diet was calculated so that the amount consumed per unit weight of animal per day should be the same at different protein values: about 0·8 mg/g body-weight.

The time of onset of signs of intoxication, namely reduced food intake, roughening of the coats and stiffness of the limbs was noted. Radiographs revealed exostoses after 21, 12, 9, 12 and 15 days in rats fed food containing toxin at protein values of ND-p Cals = 10·1, 6·2, 4·6, 3·7 and 2·1% respectively. Rats fed diet of ND-p Cals = 4·6% restricted to 75% *ad lib.* intake i.e. an effective ND-p Cals = *c.* 1, did not show any sign of intoxication during the period in which they survived (15–17 days). Only two rats which were still alive after 21 days showed rarefaction of bone together with slight exostoses.

Osteolathyrism, therefore, developed earliest in rats fed on diets having a protein value of ND-p Cals about 4·6% and less rapidly as the protein value was increased

to ND-p Cals = 10·1 or was reduced to ND-p Cals = 2·1%. No complete explanation of these results can be offered; however, they do suggest that in tests for toxicity attention should be paid to the protein value of the diet of the consumer.

We are indebted to Abbott Laboratories Ltd for a generous supply of β-aminopropionitrile fumarate.

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The effects on the newborn rat of force-feeding carbohydrate for 3 days during pregnancy. By K. HALDER and B. S. PLATT, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

In a recent review (Kalter & Warkany, 1959) of the role of nutritional disturbances during pregnancy in the production of congenital malformations, there is little information on the effects of protein. To study the effect of acute protein deficiency of the mother on the foetus, five groups of pregnant rats (1, 2, 3, 4, 5) were force-fed non-protein diets for 3 consecutive days coinciding with the 4–6, 7–9, 10–12, 13–15 and 16–18 days of gestation for the successive groups. Each period of forced feeding was preceded and followed by feeding stock diet *ad lib.* The rats were killed on the 21st day of pregnancy, the litters removed and examined. There were no obvious abnormalities in litter size, foetal and placental weights, or malformations. Histological examination revealed deposition of glycogen in the livers of the foetuses of groups 1, 2, 3 and 5 as in normal newborn pups, while those of the litters of the fourth group showed much less glycogen. The litters of groups 1–3 showed normal β-cells in the islets of the pancreas, filled with stainable granules and adrenal cortices with normal lipid spaces, whereas in the litters of groups 4 and 5 the β-cells showed very few granules and the adrenal cortices had increased lipid spaces. Litters of control pregnant rats force-fed the same quantities of the complete diet (replacement of 15% starch by casein) on the same days as groups 4 and 5, showed no abnormalities.

Changes can therefore occur in some organs of the newborn rats if the nutritional injury to the mother during pregnancy coincides with the time of maturation of the organ. In foetal rats the maturation period for hepatic glycogenic activity is 14–16 days of gestation (Corey, 1935); for the β-cells (Hard, 1944) and the adrenal cortex it is 13–19 days (Pankratz, 1931; Jasomovitch, Ladman & Deane, 1954).

Severe protein deficiency of the mother during pregnancy, even for a short period, adversely affects some developing organs of offspring superficially normal.

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The buccal mucosa in pigs on a protein-deficient diet with and without added carbohydrate. By B. T. SQUIRES, *Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Changes in human buccal mucosa in malnutrition have already been described (Squires, 1958, 1961); similar changes have now been observed in epithelial smears from pigs on a low-protein diet (LP) (Friend, Heard, Platt, Stewart & Turner, 1961) given alone or with a daily supplement of carbohydrate (LP + CH). The experimental details were as follows: pig A received the 20 CLP diet (i.e. the LP diet with 20 g starch replaced by an equal amount of casein for every 100 g diet) and showed 10–15% of abnormal cells throughout the experimental period of 133 days; pig B, which received the LP diet throughout, showed a steady rise, until on the 133rd day 60% of cells were abnormal; pig C received the LP diet for 93 days and showed a steady rise to 42%; it was then given the 20 CLP diet, and after a further period of 3 weeks the proportion of abnormal cells had fallen to the level shown by pig A. Pig D was fed on the LP+CH diet for the first 63 days and showed an increased and more rapid rise to 76% by this time; the carbohydrate supplement was then omitted, followed within a week by a reduction of abnormal cells to 33%; 2 weeks later the diet was changed to 20CLP, after which the proportion of abnormal cells fell rapidly to that shown by pig A. It appears, therefore, that not only was the LP diet associated with a marked, but reversible, change in the appearance of the buccal mucosal cells, but also that this change was exaggerated by the addition of carbohydrate to the LP diet; exaggerated responses in other tissues have been previously observed by Heard, Platt & Stewart (1958).

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The oxygen consumption of sheep when walking in cold and warm environments. By J. L. CLAPPERTON, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Two closely shorn wether sheep were given a submaintenance ration of hay and were confined in a respiration chamber at environmental temperatures of 28° and 17°. The former temperature was above and the latter below the critical temperature of the sheep. The sheep walked on the horizontal surface of a treadmill within the chamber at a speed of 24 m/min (0·9 miles/h) for two periods each of 120 min and

rested for a further two 120 min periods each day. Oxygen consumption was determined during the whole of each 120 min period. Each sheep was exposed to each temperature for 8 days, giving a total of 128 determinations of oxygen consumption.

Table 1. *Mean oxygen consumption of two sheep when walking and when standing at rest at two environmental temperatures*

Environmental temperature (°C)	Conditions	Oxygen consumption (ml/min) Sheep D	Sheep H
	When walking (*a*)	410·8	388·4
17	When standing at rest (*b*)	341·6	354·6
	When walking (*c*)	301·3	280·8
28	When standing at rest (*d*)	210·4	215·0
Increase in oxygen consumption due to walking at 17°		69·2	33·8
Increase in oxygen consumption due to walking at 28°		90·9	65·8

The mean oxygen consumption of each sheep is shown in Table 1. The standard error attached to the eight means in the table was $\pm$ 5·2 ml O_2/min. The increase in oxygen consumption due to walking in the cold environment (*a*–*b*) was less than that measured in the warm (*c*–*d*). This confirms observations made by Hart (1952) with non-acclimated mice. The results showed that at the lower temperature some of the heat produced during walking within the body was used to keep the animal warm. Not all the heat equivalent of the oxygen consumed during walking is liberated as heat in the body because external work is done. If the mechanical efficiency of horizontal walking in the sheep is the same as the efficiency of work of ascent namely 37% (Clapperton, 1961), and the whole of the heat liberated in the body was used to keep it warm in the cold, the apparent cost of work at 17° should be 33·6 and 24·3 ml O_2/min for sheep D and H respectively. In both instances oxygen consumption was higher than this theoretical value suggesting either that some of the heat produced was dissipated without helping to warm the sheep, or that increased losses of heat by convection occurred during walking.

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The effect of deficiencies of calcium and vitamin D on bone growth of chicks treated with sex hormones. By F. HERTELENDY and T. G. TAYLOR, *Department of Physiological Chemistry, University of Reading*

There were four experimental treatments with twelve female chicks/treatment: (1) normal calcium (1·2% Ca)–normal vitamin D (100 i.u./100 g), (2) normal

calcium–low vitamin D (5 i.u./100 g), (3) low calcium (0·2% Ca)–normal vitamin D, (4) low calcium–low vitamin D.

Sex hormones (100 μg oestradiol benzoate and 33 μg testosterone propionate/100 g body-weight) were injected daily into the pectoral muscles from 29th day of age. Some of the chicks were killed after 7 days' treatment and the rest after 10 days'.

The chicks on the vitamin D-deficient diets weighed less than half as much as those on the corresponding diets containing adequate amounts of the vitamin. The mean live weights of the birds on the Ca-deficient diets were approximately 80% of the weights of the chicks on the corresponding normal-Ca rations. Thus the deficiency of vitamin D inhibited growth to a far greater extent than the deficiency of Ca. The weights of the dry, fat-free bones were expressed as a percentage of body-weight. A simple deficiency of vitamin D reduced bone weights and body-weights to a similar extent, so that the relative bone weights of the normal Ca–low D birds were the same as the normal Ca–normal D chicks. A deficiency of Ca, on the other hand, reduced relative bone weights by approximately 20%, irrespective of the level of vitamin D in the diet. Data for the femur and tibia are shown in the table: weights of humerus, radius and ulna showed similar differences.

Mean live weights and bone weights of experimental chicks

		No. of chicks	Mean live weight (g)	Ash in femur (%)	Mean weight as percentage of live weight			
					Tibia	Femur	Femur ash	Femur matrix
Normal Ca	Normal D	10	678	55·8	0·252	0·207	0·116	0·091
	Low D	11	326	46·6	0·259	0·209	0·097	0·112
Low Ca	Normal D	10	520	45·2	0·199	0·162	0·073	0·089
	Low D	6	254	42·0	0·198	0·159	0·067	0·092

When the mean weights of ash and organic matter in the femur were calculated as a percentage of body-weight, it was found that, on a normal-Ca diet, a deficiency of vitamin D reduced the weight of ash and increased the weight of matrix. On the low-Ca diet the percentage of organic matter was similar at both levels of vitamin D, agreeing very closely with the percentage found in the normal D–normal Ca birds. The main effect of the low-Ca diets was on the percentage bone ash and a deficiency of vitamin D appears to have had a much smaller effect on bone composition than it had at the normal level of Ca.

Distribution and fatty acid composition of depot fat of pigs reared on synthetic diets with and without fat. By W. M. F. LEAT* and A. CUTHBERTSON, *School of Agriculture, University of Cambridge*, and A. N. HOWARD and G. A. GRESHAM, *Department of Pathology, University of Cambridge*

Twenty-eight Large White × Essex piglets were divided into four groups and reared from 10 lb to 200 lb live weight on the following diets: diet 1 (containing

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<0·1% fat) consisted of sucrose, casein, Cellophane, minerals and vitamins. In diets 2 and 3, 10% of either beef tallow or maize oil replaced an isocaloric weight of sucrose. Diet 4, a natural diet, containing 2·2% fat, consisted of barley, middlings, dried skim milk, minerals and vitamins. Growth rate and food conversion were similar in all groups. The animals on the natural and beef-fat diets had the best skin condition and those on the low-fat the worst.

Detailed dissection of seven selected animals showed that the pigs fed maize oil had the most depot fat and those reared on the natural diet had the least. In the animals fed on the maize oil and natural diets there was proportionately more subcutaneous fat than in those fed low-fat and beef-tallow diets. Intermuscular fat, however, was proportionately higher in the pigs fed low fat and beef tallow. Of the depot fatty acids of the pigs fed low fat and beef tallow 92% consisted of palmitic, stearic and oleic acids with linoleic comprising less than 1%. In the pigs fed maize oil over 20% of the fatty acids consisted of linoleic, mainly at the expense of oleic acid.

Major fatty acids of the perinephric fat of pigs fed various diets

	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)
Low fat (0·1% fat)	27·4	18·0	47·4	0·2
Beef tallow (10% fat)	24·0	17·0	50·5	0·5
Maize oil (10% fat)	19·5	17·1	35·5	22·3
Natural (2·2% fat)	28·8	16·1	42·1	5·1

Callow (1935) has reported that the iodine value of the back fat decreased along its length from head to tail. Detailed analysis of the fatty acid composition of depot fat from different positions along the back showed no consistent change in the content of palmitic, stearic and oleic acids although there was a small but definite decrease in linoleic acid towards the tail in pigs fed low fat or beef tallow. Inner back fat contained more stearic and palmitic acids and less oleic and linoleic acids than outer back fat under all treatments. The fatty acid composition of intermuscular fat was very similar to inner back fat, whilst that of perinephric fat was more saturated.

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Pathological and biochemical changes in pigs given semi-synthetic diets containing no fat, beef tallow and maize oil. By A. N. HOWARD, W. M. F. LEAT, G. A. GRESHAM, D. E. BOWYER and I. W. JENNINGS, *Departments of Pathology and Agriculture, University of Cambridge*

Groups of pigs were given semi-synthetic diets containing no fat, 10% beef tallow, 10% maize oil and compared with those given a commercial ration. Details are the same as those already described (Leat, Cuthbertson, Howard & Gresham, 1962). Various tissues and organs were examined by conventional histopathological techniques and the plasma and liver analysed for protein and lipids.

Arterial lesions were seen in all groups, but were most abundant and severe in those given the semi-synthetic diets. Two sorts of lesions were seen in the aorta: (a) slightly elevated foci of inner medial calcification (b) a slight degree of intimal thickening consisting of collagen and elastic fibres with some sudanophil fat. The latter type were more abundant in the pulmonary, bronchial and coronary arteries and resembled early human atherosclerosis.

Fatty changes occurred in the liver of those animals given the fat-free diet. Medium-size droplets of sudanophil lipid distended the centrilobular hepatic cells. In the beef-tallow group, diffuse fibrosis extending into the lobules from the portal tract was seen. No significant pathological changes in other organs were observed. In the plasma of the beef-tallow and fat-free groups, elevated levels of eicosa trienoic acid indicated that these groups were deficient in essential fatty acids (EFA). There were no differences in plasma cholesterol or glycerides between the groups but the total phospholipids were increased 70% in those given beef tallow and maize oil. The percentage composition of the plasma phospholipids showed considerable differences. In the EFA-deficient groups, lecithin was markedly increased, and sphingomyelin and lyso-lecithin decreased. Plasma protein was lowered 10% in the fat-free group and the albumin : globulin ratio diminished. This was considered a non-specific response to liver damage.

In the liver, there were no differences in total lipid, but the total cholesterol was increased 50% in the beef-tallow and maize-oil groups and $2\frac{1}{2}$-fold in the fat-free group. The increase was chiefly in esterified cholesterol.

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The influence of age, at the commencement of calcium deficiency, on the growth and health of rats fed upon meat. By S. G. Impey and T. Moore, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Restriction of weanling rats for a few weeks to a diet of raw minced beef, even with doses of vitamin D, leads to cessation of growth, to severe softening, deformity and fracturing of the bones, and eventually to death. The skeletal injuries can be prevented by adding 0·5% of $CaCO_3$ to the meat, even without vitamin D (Moore & Sharman, 1960*a*,*b*). In contrast subsequent experiments on mature female rats have shown that they may subsist on beef, without Ca supplements, for a year or more, or until death has resulted from old age or intercurrent disease. Although their bones usually show minor evidence of decalcification there are no fractures, gross deformities, or readily noticeable softening.

It seemed important, therefore, to study the effect of age, and initial body-weight, on the ability of rats to thrive on a diet of beef. Six groups, each of three males and

three females, were set up. All the rats were given minced beef from weaning, with adequate doses of vitamins A, D, E and K, and of copper. Ca supplements were supplied, or not supplied, over different periods to the various groups, but when given were always in the form of $CaCO_3$, as 0·5% of the diet. In group 1 (positive controls) the rats received Ca from the start of the experiment. Groups 2–6 were allowed the Ca supplement for 0, 6, 15, 23 and 34 days after the start of the experiment, and were thus first deprived of Ca at mean body-weights, for both sexes, of 42, 69, 119, 172 and 232 g, respectively. It was found that the ability of the rats to survive, and to continue to grow, depended upon the stage at which Ca was withdrawn from the diet. Thus in group 2, deprived of Ca from the start of the experiment, most of the rats grew slowly for 5 weeks, attaining a mean body-weight of only 116 g. The bones became soft, deformed, and fractured, and five of the animals died after 31–72 days. In group 3, given Ca for 6 days, growth ceased after about 6 weeks, or 5 weeks of Ca deprivation, with the mean body-weight 180 g. One rat in this group died after 44 days, and the bones of all the rats were deformed. In group 4, given Ca for 15 days, growth ceased after 6 weeks, or only 4 weeks of Ca deficiency, at a mean body-weight of 218 g. Later slow growth was resumed. In groups 5 and 6, given Ca for 23 and 34 days respectively, the rates of growth after deprivation differed but little from those observed in the positive control rats of group 1. After 53 and 42 days of Ca deprivation the mean body-weights were 300 and 340 g respectively. X-ray photographs of femurs indicated, however, that calcification was much less dense in the bones of all the rats of groups 2–6 than in the control rats.

In group 1 the faeces became bulkier and lighter in colour as the animals approached maturity: this change was not seen in the other groups.

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Copper deficiency in rats fed upon meat. By T. MOORE, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

The occurrence of copper deficiency, simultaneously with calcium deficiency, in rats fed upon meat has recently been reported. Young piebald rats, fed upon raw minced beef steak, with supplements of fat-soluble vitamins, developed hypochromotrichia and anaemia. These abnormalities were prevented, and growth was improved, by the addition to the meat of 10 μg Cu/g as sulphate (Moore, 1962). In further experiments hypochromotrichia, anaemia, and dental depigmentation were repeatedly observed in rats fed upon beef. Cu, but not manganese or pantothenic acid, prevented these abnormalities.

Rats also became deficient in Cu when they were fed upon raw minced mutton or pork. The Cu contents of these meats, kindly estimated by Dr J. M. Walshe, were 1·28 and 1·01 μg/g, wet weight, respectively. These values differ little from that

previously reported for beef, and suggest that the indications of Cu deficiency in rats fed upon various forms of meat are caused by a failure in the absorption of this element, rather than its absence from the diet. The possibility that the high phosphate content of meat may depress the absorption of Cu was strengthened by the prevention of hypochromotrichia in mature rats by the addition of 0·5% of $CaCO_3$ to a diet of beef. Calcium carbonate, however, did not give full protection against hypochromotrichia in young rats.

In weanling rats, fed upon meat without Cu, slight hypochromotrichia could usually be seen after about 3 weeks, and the greyness became pronounced after 6 weeks. In such rats normal pigmentation could be restored by the addition of Cu to the diet; the reappearance of pigmentation took about the same time as its previous loss. In rats which had been protected, by supplements of Cu, hypochromotrichia developed at about the same rate as in rats previously given a stock diet, after the Cu had been withdrawn.

In preliminary breeding experiments, mature female rats were mated, and were then kept upon: (1) a stock diet, (2) beef steak with no mineral supplement, (3) beef steak with Ca only, (4) beef steak with Cu only, (5) beef steak with both Ca and Cu. Litters were promptly produced by all the rats. All the rats given the stock diet, or meat supplemented with Cu, with or without Ca, successfully reared their litters. The pigmentation of the mothers remained normal. Of the remaining six mothers, only one rat, a member of the group given meat without Ca or Cu, succeeded in rearing its litter. Soon after the completion of its lactation, in contrast to those members of the same group which had not reared their young, this rat became thin, and its hair very grey. These findings suggest that an adequate supply of Cu may be even more important than Ca for lactation in the rat.

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The effect of level of feeding before and after calving on the concentration of plasma glucose in the cow. By J. E. Storry and J. A. F. Rook, *National Institute for Research in Dairying, Shinfield, Reading*

A full explanation of the range of values from 30 to 90 mg/100 ml reported in the literature for the concentration of plasma glucose in lactating cows has not been given. Allcroft (1933) attributed the diurnal variation in blood sugar of lactating cows to the high requirement of glucose for milk production and Reid (1950) has shown that during late pregnancy in the sheep, when the demand for glucose by the foetus is high, a reduction in food intake is associated with a fall in the blood glucose concentration. Recently we demonstrated that the intraruminal infusion of individual volatile fatty acids caused marked changes in the blood-plasma glucose concentration of lactating, but not of dry, cows (Storry & Rook, 1961) which suggests that a fine balance exists between the requirement of glucose for milk secretion and the dietary

supply of glucogenic materials. We were therefore interested in the extent to which plasma glucose concentrations during pregnancy and early lactation in the cow could be altered by the level of nutrition.

Eight Friesian cows about 8 months pregnant were used. Four were given 16 lb hay + 20 lb concentrates/day (high) and the other four 12 lb straw/day (low). After calving two animals in each group of four received 12 lb hay/day + 6 lb concentrates/gal (high) and the others 20 lb hay/day + 2 lb concentrates/gal (low). At intervals during both feeding periods blood samples were taken by cannula from the jugular vein four times a day. The mean plasma glucose concentrations for each animal during both periods are presented in the table. In one animal maintained on a low level of feeding throughout, the plasma glucose fell to a minimum of 23 mg/100 ml 6 days after parturition. There was invariably a transient rise in plasma glucose concentrations at parturition with peak values ranging from 68 to 95 mg/100 ml.

		Mean values with their standard errors for plasma glucose concentration (mg/100 ml)	
Treatment	Cow	14 days prepartum to calving	Days 2–14 postpartum
High–High	A	69·7 ± 2·50	62·7 ± 1·41
High–High	B	73·1 ± 3·21	65·7 ± 1·25
High–Low	C	72·6 ± 4·00	49·1 ± 1·21
High–Low	D	76·9 ± 2·02	60·1 ± 2·04
Low–High	E	57·8 ± 1·53	63·1 ± 4·07
Low–High	F	47·8 ± 1·02	74·6 ± 2·16
Low–Low	G	52·2 ± 2·62	49·5 ± 1·97
Low–Low	H	47·4 ± 0·29	37·5 ± 3·83

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Orally and parenterally administered magnesium in the control of hypomagnesaemia in grazing cows. By J. A. F. ROOK and J. E. STORRY, *National Institute for Research in Dairying, Shinfield, Reading*

Twenty-one Friesian cows, yielding 2–4 gal daily, were changed abruptly from stall feeding to the grazing of a perennial rye-grass sward. A sample of blood was taken from each cow daily for the following 9 days and the serum magnesium concentration determined. On day 2, the animals were divided into blocks of three with similar serum Mg concentrations and the animals in each block were allocated at random to one of three groups. One group (A) received no treatment, but on days 5, 6 and 7, immediately following the taking of the blood sample on the preceding day, animals in the second (B) were given daily a drench of 50 g MgO (30 g Mg)

and animals in the third (C) a subcutaneous injection of 80 ml of a 25% solution of $MgSO_4.7H_2O$ (2 g Mg).

Table 1. *Daily group mean values, with their standard errors, for serum magnesium concentration* (mg/100 ml)

Days at pasture	2	3	4	5	6	7	8	9
Group A	1·05±0·16	1·60±0·17	1·66±0·15	1·65±0·10	1·89±0·10	1·51±0·10	1·66±0·11	2·10±0·14
Group B	1·98±0·14	1·64±0·15	1·60±0·16	2·03±0·25	2·41±0·21	2·06±0·18	1·98±0·25	2·23±0·25
Group C	1·89±0·17	1·54±0·18	1·41±0·14	1·75±0·08	2·34±0·12	1·99±0·10	1·95±0·16	2·37±0·18

The daily mean values for each group, given in Table 1, indicate that the treatments were equally effective in the control of hypomagnesaemia; it can be inferred that the availability of the Mg of the oxide was on average 5–10%. Animals with the lowest serum Mg concentration before treatment showed, however, the biggest increase in concentration in group C but the smallest increase in group B (Table 2), demonstrating that animals which show the most marked fall in serum Mg concentration with grazing have the least ability to absorb Mg from the oxide, and possibly also from grass.

Table 2. *Mean changes, with their standard errors, in serum magnesium concentration (mg/100 ml) from day 4 to day 5, in subgroups with different serum Mg concentrations. Figures in parentheses are the number of cows*

Range in serum Mg concentration	0·90—1·20	1·21—1·70	1·71—2·20
Group A	+0·19±0·04 (2)	+0·05±0·03 (2)	−0·18±0·07 (3)
Group B	+0·13±0·08 (2)	+0·54±0·14 (2)	+0·58±0·13 (3)
Group C	+0·45±0·08 (2)	+0·41±0·06 (3)	+0·09±0·15 (2)

Depression of blood glycerides and milk-fat synthesis by glucose infusion.

By G. L. McClymont and S. Vallance, *Department of Biochemistry and Nutrition, School of Rural Science, University of New England, Armidale, New South Wales, Australia*

Intravenous glucose infusion of cattle has been shown to depress the milk-fat percentage (Vallance & McClymont, 1959). Blood lipid analyses (method of Garton & Duncan, 1957) have now shown that this depression is associated with depression of the glyceride plus free fatty acid (FFA) fraction of the arterial blood and of the mammary arteriovenous difference (Table 1), that FFA are not utilized by the gland (mean arterial and mammary vein levels of eleven samples, 5·18 and 5·10 mg/100 ml plasma), and that the depression of FFA by glucose infusion (2–3 mg/100 ml plasma) cannot account for the depression in blood lipids. Although acetate and/or β-hydroxybutyrate metabolism may also be affected, it is concluded that the glucose-induced depression of milk-fat synthesis probably involves the following chain of events:

Increased insulin production —> depression of the rate of release as FFA of long-chain fatty acids synthesized or deposited in the adipose tissues —> depression of the rate of hepatic synthesis of blood glycerides (evidence leading to these conclusions is covered in the review of Olson & Vester, 1960) —> the rate of removal

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Table 1. *Summary of data from one cow*

	Pre-infusion	During glucose infusion (48 h)	Post-infusion
Rate of milk secretion (ml/h)	545	527	501
Milk fat (%)	4·5	2·1*	6·2
Blood glucose (mg/100 ml)	45	105 (180→60)	43
Plasma glycerides plus FFA (mg/100 ml)			
Arterial	54	37*	46
Mammary	44	33	35
a-v difference	10	4*	11

All values are means of four to eight observations at 3–6 h intervals. Milk samples were taken with aid of oxytocin.

* Significantly different, $P<0.05$, from pre- and post-infusion.

of glyceride by the mammary gland exceeding the rate of supply by the liver and absorption from the intestine —> reduction in the plasma glyceride level and so of the ability of the gland to remove glyceride —> reduction in fat percentage to a level where inflow and outflow of plasma glycerides are in equilibrium.

The major effect on milk-fat synthesis of the relatively moderate depression of plasma glyceride lends support to the suggestion (Glascock & Wright, 1961) that a particular fraction of the glycerides is a major lipid precursor of milk fat, and indicates that this fraction is particularly depressed by glucose infusion.

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Lipotropic activity of casein in ducklings receiving a basal diet with or without added choline. By Jean-Marie DEMERS and Roch CARBONNEAU (introduced by R. H. COMMON), *Department of Biological Science, University of Montreal, Montreal, Canada*

The object of this experiment was to study the influence of casein and choline on the fatty infiltration of liver, haemoglobin and cholesterol levels of the serum in ducklings.

The analyses reported were made on serum and tissues from ducklings killed at the end of a feeding period of 3 weeks. White Pekin ducklings, all 4 days old when first receiving experimental diets, were distributed over fifteen groups of eight birds each. Five groups of diets containing respectively 0, 4, 8, 12 and 18% of vitamin-free casein, and for each group three different levels of choline chloride 0, 0·1 and 0·3%, were prepared in which the total methionine was kept uniform at

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0·74% by adding suitable amounts of DL-methionine. All the diets contained also equal amounts of known choline precursors.

The diets without added casein resulted in fatty infiltration of the liver. The total liver lipids averaged 21% in ducklings fed the diet without added choline, 32% and 25% respectively in ducklings fed the diet with supplements of 0·1 and 0·3% choline chloride. In the absence of casein the growth response was very poor, total gain in body-weight averaging only 35 g in 3 weeks. Supplements of 4, 8, 12 and 18% casein at the expense of the rice stimulated growth but were unable to prevent fatty infiltration entirely, total liver lipids averaging 27, 12, 10 and 8% respectively. Supplements of choline chloride had negligible effects with these different levels of casein.

All the ducklings given experimental diets developed a high cholesterol concentration in serum. Very low red blood cell count was observed in birds fed diets without added casein or with supplement of 4% casein. At levels of 8 or 12% casein, the red blood count was low in ducklings deprived of choline. Low haematocrit (less than 50% of normal) was also observed in birds fed the diets without added choline. Simultaneous additions of casein and choline to the basal diet resulted in nearly normal haemoglobinaemia and haematocrit count in ducklings. Our results suggest that casein has a slight lipotropic activity when added to a diet poor in choline and protein. On the other hand, choline appears to be unable to prevent fatty infiltration of liver in ducklings when the level of casein in the diet is lower than 8%.

This work was done with the financial assistance of the National Research Council of Canada.

Differences in the ranges of action of vitamin E substitutes. By I. M. SHARMAN and T. MOORE, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

N,N'-diphenyl-*p*-phenylenediamine (DPPD), methylene blue, 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (Santoquin), and sodium selenite, were compared with α-tocopherol for their ability to prevent certain well-known abnormalities in rats given a diet deficient in vitamin E. The lesions under examination were: (1) low body-weight, (2) abnormalities in gait indicative of muscular dystrophy, (3) rapid post-mortem autolysis in the kidneys, (4) brown discoloration of the uterus, (5) degeneration of the testes, (6) haemolysis of the erythrocytes in vitro by dialuric acid. Six groups, each of six male and six female weanling rats, were set up, and were given a basal diet of casein 25%, sucrose 50%, lard 10%, dried brewer's yeast 10% and minerals 5%. Each rat received adequate doses of vitamins A, D and K. The rats in group 1 (negative controls) received no supplement of vitamin E. Those in group 2 (positive controls) received 2 mg of DL-α-tocopheryl acetate weekly, while those of groups 3–6 received the various vitamin E substitutes as additions to their diet. Most of the rats were killed after receiving this diet for 315–335 days. One male and fifteen females, however, died before the experiment was ended, mostly from lung abscesses.

DPPD, added as 0·125% of the diet, prevented all abnormalities, except that in some of the rats the protection against haemolysis was defective or incomplete. Spectroscopic studies indicated the presence of the antioxidant in the rats' body fat. Methylene blue, 0·125%, prevented lesions 1–4. In four out of the six males the testes were protected, but no protection was given against haemolysis. As previously reported (Moore, 1953), methylene blue affected the spleen, which was considerably enlarged. Santoquin, 0·125%, gave protection against lesions 1–3; protection against brown uterus was only partial, and no protection was given against degeneration of the testes or haemolysis. Rats given selenium, 0·0001%, as sodium selenite, reached higher body-weights than the undosed controls, but the difference was not statistically significant. Abnormalities in gait were noticed in only two out of ten rats given Se, as against nine out of ten in the control rats. Se was completely ineffective against any of the other lesions. Christensen, Dam, Prange & Søndergaard (1958) have also found that, apart from hepatic necrosis, Se fails to prevent most of the lesions due to vitamin E deficiency in the rat.

Experiments by Moore, Sharman & Ward (1954) have suggested that the range of lesions which can be prevented by methylene blue is not influenced by the level of dosing. It seems probable that there are qualitative reasons, related to the roles which they can play in metabolism, for the different ranges of activity of the various vitamin E substitutes.

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Body composition of African adults. By J. P. GREAVES*, *East African Institute for Medical Research, Mwanza, Tanganyika*

Holmes, Darke, Greaves & Read (1962) concluded, after measuring the total body water of a number of groups of African subjects, and nitrogen balances in some individuals, that retention of protein is not necessarily accompanied by the retention of a fixed amount of water. They discussed this in relation to current theories of body composition, and inferred that the lean body mass (LBM) may not be of constant composition in certain cases.

Observations of N retention and measured changes in body-weight and body water, occurring over several weeks of high-protein feeding, may be utilized in calculating the changes in body composition that must have occurred if it is assumed that such changes were tending to produce an LBM of 'normal' composition, as prescribed by Behnke, Osserman & Welham (1953). Such calculations show that over the period of study the concentration of water in the LBM fell, while that of the tissue solids (the solids of tissues other than bone minerals and the fat of adipose tissue) correspondingly rose; furthermore, this was usually accompanied by a decrease of body fat.

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Confirmatory studies on two African males, about 19 years old, were carried out over 9 consecutive weeks. Bujiku gained 3·4 kg body-weight and 0·7 l. body water, and retained 213 g N; Michael gained 4·5 kg weight and 2·6 l. water, and retained 260 g N. At the start of the study they were 15·9 kg and 13·0 kg below their standard weight for height (Odier & Mach, 1949) respectively, but showed no other evidence of malnutrition. Muscle biopsies were taken at the start and completion of the study: the water content of wet muscle (deltoid) rose from 76·8% to 79·2% in the case of Bujiku, and from 76·7% to 77·5% in the case of Michael; the protein (N × 6·25) content of muscle solids rose from 78·4% to 83·0% in the former case, and from 82·6% to 86·8% in the latter.

These results will be considered in relation to the problems of protein requirements and protein stores, and prolonged N retentions in the absence of body-weight gains.

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