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ABSTRACTS OF COMMUNICATIONS

The One Hundred and Sixth Meeting of The Nutrition Society (Forty-eighth of the Scottish Group) was held in the Department of Biochemistry, University of Edinburgh, on Saturday, 2 February 1957, at 10 a.m., when the following papers were read :

Anaemia in calves. By K. L. BLAXTER and M. K. HUTCHESON, *Hannah Dairy Research Institute, Kirkhill, Ayr*, G. A. M. SHARMAN, *North of Scotland College of Agriculture, Inverness*, and A. M. MACDONALD, *Royal Hospital for Sick Children, Glasgow*

By feeding a diet of whole cow's milk supplemented with magnesium, copper and vitamin E for periods of 18–24 weeks to calves housed in wooden pens, iron-deficiency anaemia was produced. The syndrome was characterized clinically by lethargy, inappetence and consequent failure to grow, and by severe pallor of the mucous membranes. Haematologically the signs were a fall in the haemoglobin content of the blood to 4 g/100 ml. or less. The anaemia was microcytic and normochromic and there was marked poikilocytosis. The bone-marrow reaction to the anaemia was normal, and iron dosage cured the anaemia. The iron content of the tissues of anaemic calves was reduced.

Experiments with ninety-two beef calves on fifteen farms on which single-suckling rearing methods were in operation in northern Scotland showed that daily administration of 35 mg iron as the succinate resulted in a statistically significant increase in the haemoglobin content of the blood and in the mean volume of the erythrocytes. The anaemia was mild, the haemoglobin content of the blood at 40–80 days of age being 9.3 and 10.2 g/100 ml. in control and supplemented groups. Several calves on the farms, however, were very anaemic, their blood containing less than 6 g haemoglobin/100 ml.

The difficulty experienced in producing anaemia in calves compared with the relative ease with which it can be produced in pigs is related to the fact that the relative growth rate of the piglet during the first 8 weeks of life exceeds that of the calf by a factor of about 12. The dietary requirement of iron for the calf is placed at 100 mg/day.

A comparison of five levels of copper sulphate in rations for growing pigs.

By I. A. M. LUCAS and A. F. C. CALDER, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Six pigs were selected at 8 weeks old from each of eight litters, and were allocated at random one to each of six treatments. Treatment 1 pigs were fed a meal containing 5 p.p.m. copper. For treatments 2–6 very fine crystals of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (B.P.) were

added to the meal in the following percentages: 0.012, 0.025, 0.05, 0.1 and 0.2. All pigs were fed individually until slaughtered at 200 lb. Meal allowances were changed weekly using a scale, based on live weight, which rose to 7.0 lb. for a pig of 200 lb. Three pigs failed to complete the trial, but this was not due to the treatments tested.

Summary of the results

	CuSO ₄ .5H ₂ O added to diet (%)						S.E. of means
	0	0.012	0.025	0.05	0.1	0.2	
Rates of live weight gain (lb./week):							
44-104	7.7	8.0	7.9	8.5	8.4	8.6	±0.16
104-203	10.1	10.4	10.1	10.4	10.2	9.5	±0.16
44-203	9.0	9.3	9.1	9.6	9.4	9.1	±0.14
lb. food/lb. live-weight increase:							
44-104	2.74	2.65	2.73	2.46	2.55	2.41	±0.03
104-203	3.74	3.64	3.76	3.66	3.76	3.82	±0.06
44-203	3.36	3.30	3.37	3.20	3.31	3.27	±0.04
Carcass data:							
Killing-out percentage	76.0	75.3	75.8	76.0	76.1	74.4	±0.30
Thickness of fat over 'eye' muscle (cm)*	2.2	2.2	1.9	2.2	2.3	1.7	±0.06
Means and ranges of copper contents in liver dry matter (p.p.m.)	27 (21-35)	27 (19-44)	29 (21-41)	163 (37-540)	575 (103- 1456)	3085 (1706- 4668)	

*For details of where this measurement is taken see Lucas & Calder (1956).

The table shows that before 100 lb. the highest levels of copper sulphate caused the greatest improvements in rate of gain ($P < 0.05$) and feed efficiency ($P < 0.001$). For both measures most of the significant variability between treatments was associated with linear component in log copper concentration.

Between 100 and 200 lb. no level of copper supplement improved performance, and reductions in rate of gain ($P < 0.05$) and feed efficiency (not significant) with the 0.2% level suggested marginal toxicity.

Over the total experimental period rate of gain and feed efficiency were highest on the supplement of 0.05% copper sulphate, but overall treatment differences did not approach statistical significance.

Treatment 6 pigs had the smallest killing-out percentages and fat measurements. These were probably associated with slow rates of live-weight gain after 100 lb. Liver-copper values rose sharply with dietary supplements of over 0.05% copper sulphate.

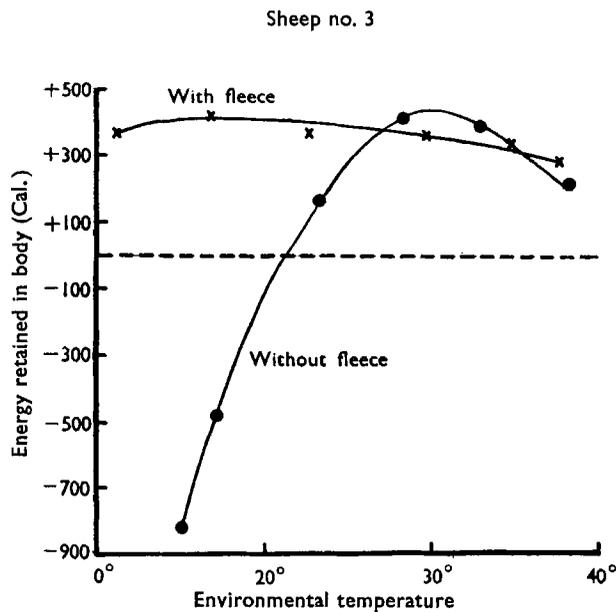
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Energy retention of sheep in relation to environmental temperature.

By K. L. BLAXTER, N. MCC. GRAHAM and F. W. WAINMAN, *Hannah Dairy Research Institute, Kirkhill, Ayr*

The energy retention of sheep was measured over 3 or 4 consecutive days at environmental temperatures ranging from 11° to 38°, using a respiration chamber in which the mean radiant temperature was identical with the ambient temperature. The sheep were given 1200 g dried grass daily in two meals at 12 h intervals and water was given *ad lib*. They were acclimatized to particular environmental conditions for 3 days before measurements were made. In two closely shorn sheep (fleece length 1–2 mm) minimal heat production and maximal energy retention occurred at an environmental temperature of approximately 30°. At a temperature of 15° heat production was increased by over 50%. The sheep then lost energy equivalent to approximately two-thirds their minimal basal heat production, despite their normal food intake. Much smaller increases in metabolism and consequent reductions in energy retention were noted when the environmental temperature was increased from 30 to 38°. The high heat production at high temperatures was associated with increased protein katabolism. At low temperatures the increase was associated with a marked increase in fat katabolism and a fall in the respiratory quotient, but no increase in urinary nitrogen excretion occurred. Experiments with the same sheep given the same amount of food when carrying a fleece indicated that the fleece (up to 10 cm thick) provided protection against temperatures as low as 12°. The acceleration of heat production at high temperatures was not appreciably greater than in sheep with minimal fleece. The results for one sheep (no. 3) are shown graphically in the figure.



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The results are interpreted to show that sheep have a considerable ability to withstand high temperatures, even when fleeced, but that their critical temperature even when fed a normal ration, if they are carrying minimal fleece, is extremely high. It appears that under these conditions of minimal insulation the critical temperature is comparable to that of naked man.

The effect of housing on the response of growing pigs to dietary supplements of antibiotic and of certain vitamins. By I. A. M. LUCAS and A. F. C. CALDER, *Rowett Research Institute, Bucksburn, Aberdeenshire*

We have reported previously (Lucas & Calder, 1955*a,b*) that weaned pigs kept to a very high plane of feeding grew as well in a cold and bad piggery as in a warmer and good piggery, but that when the plane of feeding was lowered the adverse effects of the bad piggery became evident. The rations fed contained antibiotic and unusually high levels of certain vitamins, and the experiment now reported was planned to determine whether these had affected the results obtained.

The basal diet contained 16.8% crude protein, 3.8% crude fibre, 0.9 mg riboflavin/lb. and 3.2 mg pantothenic acid/lb. It was fed unsupplemented, or with riboflavin, calcium pantothenate and ascorbic acid added at 1.2 mg/lb., 6.0 mg/lb. and 8.0 mg/lb. respectively, or with procaine penicillin added at 12 g/ton, or with the three vitamins plus antibiotic added at these same levels. The four diets were fed in both warm and cold pens with air temperatures averaging 60°F and 44°F. All pens had outdoor runs with air temperatures averaging 37°F. Eight litters each of eight pigs were used, one pig from each litter being allocated to each treatment. Feeding was twice daily to a very high plane based on live weight. Initial weights averaged 39 lb.

Eight missing values had to be calculated because of an outbreak of bowel oedema, but all other pigs remained healthy up to slaughter at 100 lb. live weight.

Procaine penicillin improved rate of live-weight gain and feed efficiency by 3% ($P < 0.10$) and 2% (not significant), and housing did not affect this response. Added vitamins were of no benefit in the warm pens, but improved rate of live-weight gain and feed efficiency in the cold pens by 5% (P almost 0.05) and 4% (P just 0.05) respectively. There was no evidence that antibiotic spared the need for extra vitamins in cold pens. In the cold pens rate of live-weight gain and feed efficiency were poorer than in the warm pens by 11% ($P < 0.001$) and 15% ($P < 0.01$), but when the extra vitamins were fed these differences fell to 7% and 10%. Killing-out percentages were slightly higher in the cold than in the warm pens ($P < 0.05$).

With data calculated on the basis of dressed-carcass weights instead of live weights the trends were the same as those reported above, but the levels of significance were higher.

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Lucas, I. A. M. & Calder, A. F. C. (1955*b*). *J. agric. Sci.* **46**, 307.

The effect of maternal social class and stature upon the incidence of prematurity. By W. Z. BILLEWICZ and A. M. THOMSON, *Obstetric Medicine Research Unit, Medical Research Council, Foresterhill, Aberdeen*

The incidence of prematurity is known to be relatively high in women of low social status and in women of small stature (Baird, 1945; Baird & Illsley, 1953; Martin, 1954). The following data show prematurity rates by height and by social class in an urban community.

Incidence of prematurity (birth weight $5\frac{1}{2}$ lb. (2500 g) or less) per 100 births. (Aberdeen legitimate, single maternities)

Registrar- General's social class	Maternal height						All heights
	4 ft. 11 in. and under	Up to 5 ft. 1 in.	Up to 5 ft. 3 in.	Up to 5 ft. 5 in.	Up to 5 ft. 7 in.	Over 5 ft. 7 in.	
I, II	7.4 (29)	5.4 (240)	3.5 (539)	1.1 (485)	1.4 (278)	4.3 (70)	4.3 (1641)
III	11.3 (542)	8.0 (1822)	6.5 (2875)	4.0 (2037)	4.1 (716)	5.6 (126)	6.3 (8118)
IV, V	13.0 (378)	9.8 (929)	7.4 (1243)	5.4 (747)	8.4 (225)	9.8 (41)	8.3 (3563)
All classes	11.7 (949)	8.6 (2991)	6.6 (4657)	4.3 (3269)	4.2 (1219)	5.1 (237)	6.6 (13,322)

Numbers of patients are shown in parentheses

In each height group, the rate rises with falling social status. In each social class, it falls with increasing stature, at least up to 5 ft. 5 in. In social class III the rate tends to rise slightly in taller women, and in social class IV and V the rise is definite. Similar trends have been found in the perinatal mortality rates of these women. The trends in the prematurity rates therefore represent differences of foetal vitality and not merely of birth weight.

The chief explanation is thought to be as follows. Where nutritional standards during childhood and adolescence are low, women do not attain the maximum stature of which they are genetically capable; the proportion of small women therefore becomes greater as social status declines. Stunting of growth is accompanied by physiological impairment which leads to increased prematurity and perinatal mortality rates. Small women in classes IV and V are those in whom stunting has been most common and most severe, and they therefore have the highest prematurity rate. Tall women in classes I and II are those in whom stunting is least common and least severe, and they have the lowest prematurity rate. The few equally tall women in classes IV and V have an innate tendency to tallness but have been reared in an environment where nutritional standards are low; this environment is more inadequate for them than for women whose growth potential is less.

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 Martin, F. M. (1954). *Med. Offr.* **92**, 263.

Rapid gastric emptying time as a cause of crying in breast-fed babies.

By JESSIE C. YORSTON and F. E. HYTTEN, *Obstetric Medicine Research Unit, Medical Research Council, Foresterhill, Aberdeen*

One hundred and six primiparae who left hospital breast feeding were followed up for 3 months; sixty-eight were worried because their babies cried excessively, and in forty-one this was an important influence in their decision to abandon breast feeding. About half the crying babies were underfed. The rest appeared on examination to be thriving and these will be considered here.

The clinical picture was characteristic. The babies settled down temporarily after feeding, but woke early for the next feed, were restless, sucked their hands, and were ravenous at the breast. Frequently the crying was associated with signs of intestinal colic, the clinical picture then being that of '3-months' colic' as commonly described. Apart from this the babies looked healthy and were gaining weight at the rate of 7-12 oz. weekly. In all of them, the symptoms of 'hunger' disappeared when bottle feeding was instituted. The change was dramatic; the baby who had been 'difficult' and 'never satisfied' on the breast became placid and happy on the bottle. The syndrome as described by the mothers was indistinguishable from that of underfeeding.

Barium sulphate was added to a bottle feed of one mother's breast milk; with her baby under the X-ray screen the mixture was seen to pass immediately into the duodenum and then rapidly through the jejunum; the whole feed was well down the small intestine within about 20 min. One week later, at the same time, the experiment was repeated with barium sulphate added to a bottle feed of dried-milk mixture; this passed normally through the pylorus after the usual interval; there was still some barium in the stomach after 2 h.

Another baby with persistent and severe colicky crying had settled down completely after weaning at the age of 6 weeks on to five feeds daily of 5½ oz. of a National Dried Milk mixture. As an experiment, 3 weeks after weaning, this baby was given feeds of pooled breast milk. Colicky crying and 'hunger' recurred immediately and he was given a total of 40 oz. of the pooled breast milk in six feeds between 2 p.m. and 6 a.m. to keep him quiet. He became contented again when given his usual feeds of cow's milk.

It is suggested that some babies have a relative hypermotility of the gut and the fine curd of breast milk passes rapidly through the stomach, leaving it empty soon after feeding.

Social differentials in the heights and weights of Edinburgh school-children 1952-6. A preliminary communication. By J. G. THOMSON and W. N. BOOG WATSON, *Public Health Department, City of Edinburgh*

Average heights and weights of Edinburgh Education Authority schoolchildren in 1955-6 are similar to the London figures for 1938 for the 5-year-old group, and to the 1949 London figures for 9- and 13-year-old groups.

There are, however, wide differences in nutrition, as assessed by height and weight measurements, between different schools in Edinburgh. Moreover, large numbers of Edinburgh children attend fee-paying schools outside the control of the Local Education Authority and are not included in this comparison.

The heights and weights of Edinburgh Education Authority schoolchildren have therefore been analysed, according to the school attended, for the years 1951-2 to 1955-6 and tabulated separately for each school, each age group and each sex. For 13-year-old boys the classification of schools was:

A: where heights and weights are around or over 62 in. and 100 lb.

B: where heights and weights are around or over 60 in. and 95 lb.

C: where heights and weights are around or under 59 in. and 90 lb.

Of seventeen Education Authority Secondary Schools in Edinburgh, all seven Senior Secondary Schools, taking children of higher I.Q., fall into category A, one Senior Secondary (Commercial and Technical) and three Junior Secondary Schools into B, and six Junior Secondary Schools into C. The two schools with the poorest height-weight records are located in the two wards of the city with the worst vital statistics.

The height and weight differentials between the 13-year-old boys of the school with the best 5-year record and those with the worst is approximately $2\frac{1}{2}$ in.- $2\frac{3}{4}$ in. and 9-10 lb. In the case of 13-year-old girls the corresponding differential is $2\frac{1}{4}$ in.- $2\frac{1}{2}$ in. and about 10-12 lb.

In the primary schools serving these secondary schools, both boys and girls at 5 years of age show a difference of 2 in.- $2\frac{1}{4}$ in. and about 5 lb. between the worst and the best. At 9 years the difference is $3\frac{1}{4}$ in.- $3\frac{1}{2}$ in. and 9-10 lb.

Ignorance of food values and bad family budgeting against a background of unsatisfactory feeding habits in the community group are probably at least as important as economic factors and present a challenging problem for the health educator.

The One Hundred and Ninth Meeting of The Nutrition Society was held at the Royal College of Surgeons of England, London, W.C.2, on Friday, 3 May 1957, at 10 a.m., when the following papers were read:

The estimation of the dilution of tritium-labelled water of low activity in body fluids in man. By J. DONE and P. R. PAYNE (introduced by B. S. PLATT), *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Tritium is a very suitable label for water in human subjects, but doses of the order of 2-3 mc have been required (Pinson, Anderson & Lotz, 1951; Prentice, Siri, Berlin, Hyde, Parsons, Joiner & Lawrence, 1952; Bradley, Davidsson, MacIntyre & Rapoport 1956; Langham, Eversole, Hayes & Trujillo, 1956). However, accurate assays are possible following doses of 0.15 mc by adaptation of procedures which have been in use for several years in our Unit for the routine assay of tritium in organic samples

(Done & Payne, 1956). The total radiation dose is then 15 mR i.e. about 15% of that received per annum from background sources. 0.03 mc would be sufficient for estimation of total body water. Results for an early period of an experiment are given in Fig. 1.

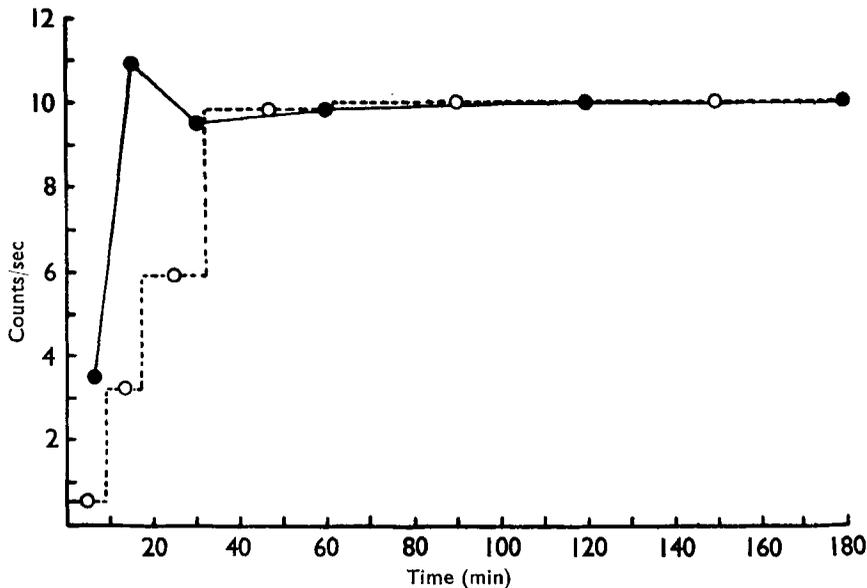


Fig. 1. Time curves of tritium in blood (—●—) and urine (· · · ○ · · ·) of human subject (70.7 kg) after injection of 0.15mc of tritium-labelled water (200 ml.).

Total body water as estimated by the dilution of the active water after 2 h was 48.6 l. (68.7% of the body-weight). Accurate observations were possible both before and after the maximum counts at the period of 'equilibration'. Values for the half-life of body water, in days, calculated after assay of later samples were as follows, figures in parentheses being the times in h at which samples were taken: 5.1 (4-24); 5.0 (24-48); 9.9 (48-96); 9.0 (96-1296). The lower figures correspond to a period (4-48 h) during which fluid intake was raised.

Body-water estimations were made on a group of ten swimmers whose weights ranged from 72.3 to 102.9 kg. The mean body-water percentage by weight was 57.7, S.D. = 2.79.

Recent modifications in application of the method to the assay of water samples will be mentioned and it is hoped to give a detailed description elsewhere.

We are very grateful to Dr R. H. Fox, Division of Human Physiology, M.R.C. Laboratories, Hampstead, for his collaboration.

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Chronic protein malnutrition in the rat: reproductive performance.

By B. S. PLATT and P. JOAN MILLER, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Rats fed on a diet poor in protein (basal 'Gambia type' diet) have, by comparison with animals on a good stock diet, fewer and smaller litters of pups (of lower birth weight) in which there is a high mortality rate in early life. Late weaning of such offspring from the breast increases survival remarkably. Some data have been published recently (Platt, 1957): the figure reproduced here supplements this statement.

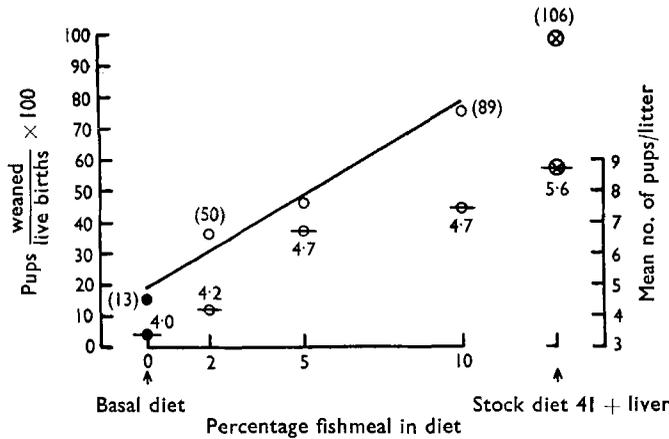


Fig. 1. Effect on percentage rats weaned, mean number of pups/litter, total number of rats born and birth weight in g, of increasing amounts of fishmeal, ○, compared with performance on basal ('Gambia type') diet, ●, and on stock diet, ⊗.

○ Percentage animals weaned. Adjacent figures, e.g. (50) show total number of rats born.

⊗ Mean number of pups/litter. Adjacent figures, e.g. 4.2, show mean birth weight in g.

Note: Results from six rats. Each produced two litters except for rats on basal diet when only one rat produced two litters, two rats produced one litter each and one was infertile.

The fishmeal employed in these experiments had a biological value of 60 (kindly determined for us by Mr D. S. Miller).

REFERENCE

Platt, B. S. (1957). *Human Protein Requirements and their Fulfilment in Practice*. Proceedings of a Conference in Princeton, United States (1955), sponsored by FAO/WHO/Josiah Macy Jr. Foundation, p. 56. [J. C. Waterlow and Joan M. L. Stephen, editors.]

Protein malnutrition in the pig. By C. B. KNOWLES (introduced by B. S. PLATT), *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Work is here described which shows the baby pig to be very suitable for investigation into the consequences of protein shortage.

The diets contained 4.5–10% of protein, mainly from wheat with contributions from haricot beans and grass meal. A salt mixture, 2% arachis oil containing ergo-calciferol (50 i.u./g) and maize starch completed the diets. They were provided *ad lib.* in the dry powdered state. All animals were dosed with iron at about a week old.

In one experiment, six animals were weaned on to diets at 13 days of age. The four on diets containing 4.5 or 6.5% protein failed to gain weight and after some 3–4 months had signs similar to those described for severe protein malnutrition in infants. Prominent amongst these were weakness, apathy, hypochromotrichia, anaemia and oedema. The skin was dry, desquamating and fissured. Death occurred after 4–6 months with intermittent coma over the last few days of life. Diarrhoea was absent until shortly before death. At autopsy, free fluid was found subcutaneously, intraperitoneally and even intramuscularly. Total plasma protein was less than 4 g/100 ml. with an albumin : globulin ratio of around 0.3 (magnesium-sulphate precipitation of globulins). The two animals on the 10% protein diet were much less affected and continued to grow slowly.

In another group of animals on 5.5% protein, total plasma protein, plasma amylase, haemoglobin concentration and packed red-cell volume were found to be significantly lower than in a group of litter-mates on 10% protein.

There was little or no diuretic response in 3 h at any of the protein levels to an oral water-load of 5% of body-weight. This agrees with the observations of Heller & Blackmore (1953) and others on protein-depleted rats. After two animals which had received a 10% protein diet for 5 months had been given a good diet for 1 week, a normal response to a water load was present.

Animals live much longer than 6 months if the low (4.5–6.5%) protein diets are not given until a later stage of development. Cataracts have been noticed in such animals at about 12 months.

There appears to be a somewhat critical stage, which for a given breed is more closely related to growth (weight) than to age, before which it is desirable to start feeding the low-protein diet in order to secure a satisfactory preparation in a few months and of a still convenient size.

REFERENCE

Heller, H. & Blackmore, K. E. (1953). *Brit. J. Nutr.* **7**, 349.

Level of protein in the diet and apparent nitrogen digestibility in the pig.

By P. L. PELLETT, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

The use of chromic oxide (Cr_2O_3) in digestibility trials has been described by Dewey & Platt (1954) who give references to earlier work. In the present work Cr_2O_3 was determined by the method of Bolin, King & Klosterman (1952). Nitrogen was determined in the same digest, an aliquot being made strongly alkaline and distilled in a Markham apparatus. Somewhat low values were obtained for nitrogen but these

affected the value obtained for the apparent nitrogen digestibility by less than 1%.

The concentration of the chromic oxide in the diets was approximately 0.3% and faeces were collected for analysis after at least 1 week upon the diet, duplicate samples being taken after a further 2 days. The animal used for the experiment was a pig which had been on a 4.5% protein diet for over 6 months. The results obtained show that when the protein content of the diet was increased to 7.5% the apparent nitrogen digestibility rose immediately from 56% to 80% but that when the animal was returned to the 4.5% protein diet the apparent digestibility was maintained

The apparent digestibility of nitrogen in a protein-depleted pig

Time (weeks)	1	2	3	4	5	7	8	9	11
Protein in diet (%)	4.5	4.5	7.5	7.5	4.5	4.5	4.5	4.5	4.5
Apparent digestibility of nitrogen (%)	56	56	80	84	80	73	71	66	58

at a high level for several weeks before returning to the previously obtained value of 56%. It is suggested that the period upon the high level of protein allowed the development of increased amounts of digestive enzymes to occur and that this effect persisted for several weeks. It is of interest that in the week before the animal's death the apparent nitrogen digestibility had fallen to 30% upon the low-protein diet.

Low apparent nitrogen digestibility values have been reported for undernourished young Gambian children (Platt, 1954).

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Prolidase and nitrogen content of intestinal mucosa in normal and protein-deficient animals. By C. R. C. HEARD (introduced by B. S. PLATT), *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

The effects of protein malnutrition on the liver and pancreas have been extensively studied and it is known that the pancreas is affected before the liver. Although it is very probable that the intestine is affected at an even earlier stage, very little attention has been given to the effect of low protein supplies on intestinal enzymes (Platt, 1954). Prolidase was selected for study because of indications that the enzymes hydrolysing proline-peptide linkages may be confined to the intestinal portion of the alimentary tract. Prolidase is present in many tissues, including the small intestine but is absent from the pancreas and may therefore be regarded as an enzyme typical of the intestinal stage of digestion.

Aqueous glycerol extracts of washed mucosa of the whole intestine were assayed as described by Smith & Bergmann (1944) except that the proline was estimated

either by the isatin reaction on paper (Smith, 1953) or by a ninhydrin colorimetric procedure based on that of Troll & Lindsley (1955). The nitrogen content of the mucosal extracts was also estimated. Enzyme activity (K), nitrogen content (E) and prolidase concentration in terms of nitrogen ($C = K/E$) were calculated.

Animals	Diet	Age (days)	Weight (g)	Weight ² (g)	K	E	C
Rat (1)	Protein-free (45 days)	95	26	11	0.02	0.9	0.027
Rats (4)	'Gambia type'	85	34	14	0.35	10.7	0.034
Rats (4)	Diet 41 and liver	85	142	41	1.05	28.8	0.036
Pigs (2)	Newborn	0	1105	192	10.75	216.0	0.052
Pigs (2)	4.5% protein	130	3600	465	15.00	274.0	0.056

Typical results are shown in the table. Even severe protein malnutrition does not appear to alter the concentration of intestinal prolidase which remains at the level typical for the species. In chronic malnutrition in rats the total prolidase and total nitrogen for the whole small intestine are closely related to the size of the animal and more particularly to the animal's protoplasmic mass as represented by the three-fourths power of body-weight. For its size the malnourished animal is as well provided with intestinal enzymes as its much larger age control. However, in an animal with extreme protein depletion there is a real loss of intestinal enzymes.

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The utilization of carbohydrates in the young pig. By A. M. DOLLAR, K. G. MITCHELL and J. W. G. PORTER, *National Institute for Research in Dairying, Shinfield, near Reading*

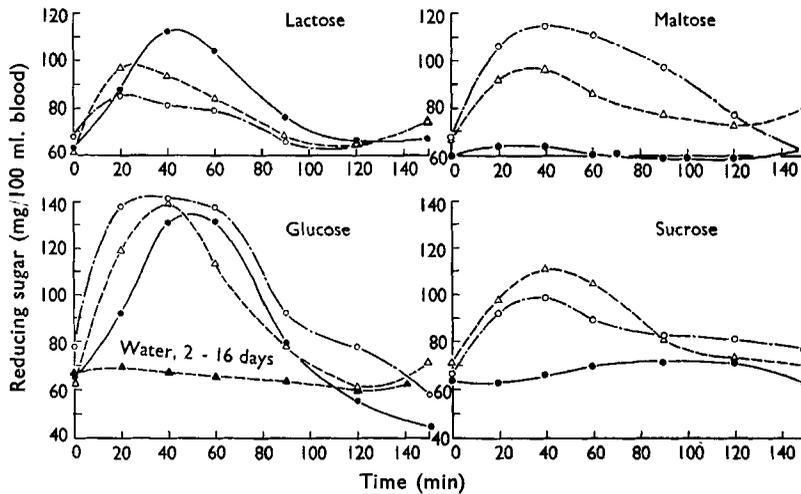
If young calves and pigs can utilize cheap starch-containing foods in milk-replacement diets, their use would be of practical value. Dollar & Porter (1957) by following changes in the level of the reducing sugars in the blood showed that the young calf cannot utilize maltose, dextrin or starch for the first 4-5 weeks of life, and sucrose for at least 7 weeks.

In the present experiments five or six pigs from each of three litters were removed from the sow at intervals of 6-10 days and fasted for 3-7 h before receiving by mouth water or an aqueous solution of glucose, lactose, maltose, sucrose or dextrin (0.82 g/lb. body-weight). Immediately before dosing and at intervals during the following 2.5 h, 0.05 ml. blood was taken from an ear vein. The concentration of reducing sugar in the blood was measured by Somogyi's (1945) method. Results with water, glucose, lactose, maltose and sucrose are shown in the figure; findings with dextrin were similar to those with maltose. It is clear that the newborn pig can utilize, of the sugars tested, only lactose and glucose. However, ability to assimilate sucrose, and maltose and dextrin develops during the first 10 days of life. Pigs

from two of the litters showed a decreased utilization of lactose after 2-3 weeks; pigs from the third litter still utilized lactose when 36 days old.

These findings agree with those of Kitts, Bailey & Wood (1956) and Bailey, Kitts & Wood (1956) who studied the changes in the amylase, lactase, maltase and sucrase activities of the pancreas and intestinal mucosa of the young pig.

Means for three litters; ●—●, 2-4 days; △---△, 9-11 days; ○—○, 15-22 days



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Biological activity for the rat of strepogenin-like peptides in partially hydrolysed proteins. By E. KODICEK and S. P. MISTRY*, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Strepogenin (Sprince & Woolley, 1944), a peptide-like factor in partially hydrolysed proteins, has a growth-stimulating effect for certain bacteria. There is growing evidence that a number of peptides, rather than one single specific factor, are involved, and that their activity is due, at least in part, to a utilization superior to that of their component amino-acids.

It has been reported that rats or mice require a source of strepogenin (Woolley, 1945), since diets containing synthetic amino-acid mixtures or completely hydrolysed casein as the only source of nitrogen, did not support growth to the same extent as intact proteins (Womack & Rose, 1946), and supplements of partially hydrolysed, strepogenin-containing proteins increased the growth rate significantly (Woolley, 1946; Ågren, 1947). In dogs, however, no such effects were observed (Silber, Howe, Porter & Mushett, 1949). Even in rats and mice, other workers

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have not observed a 'strepogenin' effect of partially hydrolysed proteins (Frost & Sandy, 1948; Maddy & Elvehjem, 1949; Ramasarma, Henderson & Elvehjem, 1949).

In view of these contradictory findings, we have reinvestigated in three series of experiments comprising eighty-eight rats, the effect of supplements of partially hydrolysed proteins. Rats were given a strepogenin-free diet containing 20–26% completely hydrolysed casein, to which were added 0.2% DL-tryptophan, 0.2% L-cystine and 0.2% DL-methionine, adequate vitamins, salts, 64–71% sucrose and 3% cottonseed oil. The supplements tested for growth-stimulating activity were partially acid-hydrolysed insulin and bovine plasma albumin (Armour & Co.) and proteolysed liver powder (Hepamino, Evans). They were supplied in the rat diet, at the expense of the strepogenin-free casein hydrolysate, at levels of 1%, 3–6% and 0.7%, respectively. These levels were chosen on the basis of their relative strepogenin potency as determined by *Lactobacillus casei* assay (Kodicek & Mistry, 1952), namely insulin 100% activity, bovine plasma albumin 45%, Hepamino Evans 20%, casein 2%. Casein or bovine plasma albumin, both completely hydrolysed, had no microbiological activity.

The non-supplemented groups, receiving the strepogenin-free casein hydrolysate, showed a suboptimal rate of weight gain and a low food utilization (64–90% of that of control rats given intact casein). None of the three partially hydrolysed protein supplements, however, produced an improvement. The better performance of rats given intact casein cannot be attributed to strepogenin peptides, since none of the partially hydrolysed supplements gave a positive response, despite their high strepogenin activity for *Lb. casei*. The inferior potency for rats of completely hydrolysed casein, compared to intact protein, might possibly be due to an imbalance of amino-acids caused by destruction or isomerization during hydrolysis, with a consequent adverse effect on growth (Elvehjem, 1956).

We wish to thank Armour Co. Ltd for a generous gift of bovine plasma albumin.

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Effect of cortisone on toxicity of vitamin D₂ in rats. By E. M. CRUICKSHANK and E. KODICEK, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Pathological conditions in man associated with hypercalcaemia, e.g. sarcoidosis (Anderson, Dent, Harper & Philpot, 1954), and idiopathic hypercalcaemia of

infants (Creery & Neill, 1954; Macdonald & Stapleton, 1955), have been attributed to the toxic effects of vitamin D, either as the result of overdosage or of hypersensitivity to the vitamin. Since cortisone treatment effectively alleviates the clinical symptoms, lowers the hypercalcaemia and decreases the intestinal absorption of calcium, as it does also in typical hypervitaminosis D (Fanconi, 1956; Winberg & Zetterström, 1956), it has been postulated that cortisone might act as a structural antimetabolite towards vitamin D (Anderson *et al.* 1954).

To test the possibility of a direct interaction between vitamin D and the hormone, we have studied the effect of cortisone acetate (COAC) on hypervitaminotic rats. Twenty-four animals, weighing about 70 g, were divided into three groups and given for 5 weeks the Steenbock rachitogenic diet 2965. The rats in group 1 received, *per os*, 1 mg vitamin D₂ daily in arachis oil; group 2 received in addition 1 mg COAC intramuscularly daily. The rats in group 3, which served as controls, were injected daily with 1 mg COAC and given a maintenance dose of vitamin D₂ (1.25 µg/week).

Cortisone administration did not improve the condition of the hypervitaminotic animals; on the contrary the COAC-treated animals lost more weight (−18g) than the untreated (−6g) and their mortality was higher (75% compared to 13%). The control rats, receiving COAC only, continued to increase in weight (13 g), and none died. Their daily urinary phosphorus excretion was 0.14 mg/rat, and bone ash was 48%; no metastatic calcifications were observed. In the hypervitaminotic animals, irrespective of whether COAC was given or not, there was an increased urinary P excretion (2.7–3 mg/rat), a slightly lower bone ash (41–43%), and excessive calcifications in the kidneys and aorta.

COAC treatment had therefore no beneficial effect in hypervitaminosis in the rat, in contrast to the positive findings in man. The results imply that there is not a direct antimetabolite competition between cortisone and vitamin D. The undisputed beneficial action of COAC in human conditions would thus be exerted by affecting indirectly the metabolic changes produced by the toxic effects of vitamin D. The ineffectiveness of COAC in rats might be due to species differences. It will be noted that, in our experiments, the ratio of COAC to vitamin D was smaller than that usually used in man, but it was not feasible to test the effect of higher levels because of the known toxic effect of COAC on rats (Follis, 1951).

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Vitamin D₃ toxicity in calves. By P. S. BLACKBURN, K. L. BLAXTER and ELIZABETH J. CASTLE, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Experiments with calves given rations of whole milk supplemented with iron, magnesium and α -tocopherol, showed that as daily dosage with vitamin D₃ increased from 0.5 to 4.0×10^6 i.u., serum calcium increased, serum Mg fell, and growth was progressively retarded. Clinical and post-mortem signs of hypervitaminosis, also increased progressively. Further experiments with twenty-four calves were then made in which the calves were given 1.0×10^6 i.u. vitamin D₃, or supplements of CaCO₃ or CaHPO₄, or the mineral supplements with the vitamin. The CaCO₃ supplement was 6.5 g/l. milk and the CaHPO₄ supplement 8.8 g/l. Both supplements raised the Ca content of the milk from 1.2–1.3 g/l. to 3.8–3.9 g/l.

The serum Ca of calves given CaCO₃ increased above that of controls given none. Addition of vitamin D₃ caused a further increase to values greater than those found with vitamin D₃ alone. When CaHPO₄ was given, the serum Ca was unchanged and when vitamin D₃ was given in addition, the serum Ca was found to be lower than that in calves given the vitamin alone. Serum Mg was depressed by all four treatments, the depression being small when CaHPO₄ was given alone. An increase in the inorganic P content of whole blood occurred when CaHPO₄ plus vitamin D₃ was given. High terminal values of the non-protein nitrogen content of blood were found in three calves given vitamin D₃ and mineral supplements.

Vitamin D₃ administration led to depression of growth associated with diarrhoea, distress on exercise, a jugular pulse, and stiffness with difficulty in standing. No abnormality was found in calves given additional minerals alone. Calves were destroyed when signs were severe or when 56 days had elapsed. The mean ages at death for calves on different treatments were for those receiving vitamin D₃ 47 days, for those receiving CaCO₃ + vitamin D₃ 17 days and for those receiving CaHPO₄ + vitamin D₃ 37 days.

The pathological signs *post mortem* were gross metastatic calcification particularly of the vascular system and especially noticeable at the aorta base. The kidneys were usually pale and enlarged, and sometimes the muscles were calcified. The Ca content of soft tissues was increased very considerably in all animals receiving vitamin D₃, but not in controls or in those receiving a CaCO₃ supplement only. When CaHPO₄ was given alone a slight increase occurred. The Mg and P contents of soft tissues were also increased when vitamin D₃ was given.

Histologically, calcification of the heart was most frequent in the coronary artery and its branches, but also occurred under the endothelium and between muscle bundles. The kidneys contained cortical deposits of Ca in the vessels and glomeruli. In the medulla, calcification of blood vessels and tubules was seen. One calf given CaHPO₄ without vitamin D₃ showed calcified nodules in the left auricle on post-mortem and histological examination.

The energetic efficiency of fat synthesis from mixtures of steam-volatile fatty acids in sheep. By D. G. ARMSTRONG, K. L. BLAXTER, N. McC. GRAHAM and F. W. WAINMAN, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Twenty-eight experiments with four sheep were made. Each sheep was given a constant ration of 900 g/day dried grass throughout. In addition, either 6 l. of dilute salt solution or 6 l. of this solution containing one of two mixtures of steam-volatile fatty acids were put through a fistula into the rumen every 24 h for 7 days. One mixture contained acetic acid, propionic acid and *n*-butyric acid in the molar proportion 7.5 : 1.5 : 1.0 (the high acetic-acid mixture) and the other contained the same acids in the molar proportion 0.83 : 1.5 : 1.0 (the low acetic-acid mixture). Both mixtures were given in amounts that supplied either 450 or 900 Cal. daily. The metabolism of carbon, nitrogen and energy was measured during the final 4 days of each 7-day period. Heat increments were determined as the difference between the heat production when the acids were given and when they were not given.

Addition of 900 Cal. of the high acetic-acid mixture to the ration reduced methane production and increased the loss of energy in faeces. This alteration in the metabolism of the basal ration of grass invalidated computation of the heat increment in this instance. When 450 Cal. of the high acetic-acid mixture or 450 or 900 Cal. of the low acetic-acid mixture were given, no change in the faecal losses of energy occurred but small falls in CH₄ production took place. However, disturbance of the metabolism of the basal ration in these experiments was not sufficient to vitiate computation of heat increment.

When the salt solution was given without acids the mean energy retention was +100 Cal./24 h. The acids were therefore used to effect net synthesis. When 450 Cal. and 900 Cal. of the low acetic-acid mixture were given the increments of heat were 41.4 ± 1.8 and 42.1 ± 1.8 Cal./100 Cal. acid mixture respectively. When 450 Cal. of the high acetic-acid mixture were given the heat increment was 68.2 ± 1.8 Cal./100 Cal.

When the same mixtures were given to fasting sheep in amounts insufficient to produce deposition of fat, the heat increment of the low acetic-acid mixture was 12.8 ± 1.8 Cal./100 Cal. and of the high acetic-acid mixture 14.4 ± 1.8 Cal./100 Cal. (D. G. Armstrong, K. L. Blaxter & N. McC. Graham, 1957, unpublished). The results show that when the acids are metabolized to effect fat synthesis they are less efficiently used than when they are oxidized to meet the energy demands of maintenance. They show also that, whereas in the fasting animal efficiency of utilization was independent of the composition of the mixture given, in fattening, efficiency fell when the proportion of acetic acid in the mixtures was increased.

A study of the relationship between nutrition and reproduction in identical twin bulls. By T. MANN and L. E. A. ROWSON, *A.R.C. Unit of Reproductive Physiology and Biochemistry, Moltano Institute and Department of Veterinary Clinical Studies, University of Cambridge*

Underfeeding has long been known to affect adversely the reproductive capacity of the male, chiefly by lowering the endocrine activity of the testes and delaying sexual maturation. In the past, however, the assessment of these effects was based almost exclusively on anatomical and histological examinations of the male gonads and accessory organs, thus necessitating sacrifice of the experimental animal. The recent introduction, however, of analytical methods of semen appraisal makes it possible to assess the function of the testes and accessory organs by more quantitative methods, and what is more, in the living animal, thus obviating the need for autopsy.

The present study had as its purpose to ascertain the effect of reduced food intake on the onset of secretory activity in the accessory organs and on the appearance of spermatozoa in young, maturing bull calves. Three pairs of identical twin calves were used, one twin being reared on a normal plane of nutrition and the other on a low plane of reduced food intake. Semen was collected by the electroejaculation method. Fructose and citric-acid levels in semen served as indicators of the secretory function of the seminal vesicles, sperm density as criterion of the gametogenic activity of the testes.

Underfeeding produced a delay of several months in the appearance of fructose and citric acid. This delay was due to the absence of adequate stimulation of the testes by the gonadotrophic hormone and, consequently, to lack of androgenic stimulation of the seminal vesicles. Injection of gonadotrophin (luteinizing hormone) caused an immediate appearance of fructose and citric acid in semen.

Restricted food intake had also a definite though much less pronounced effect on sperm production. Moreover, the deleterious effect of inadequate nutrition on sperm production persisted for a long time so that even at the time of full maturity, the low-plane bull produced ejaculates with much lower sperm density than the high-plane twin.

Nutritive value of bread protein fortified with amino-acids. By A. E. BENDER, *Bovril Ltd, 148 Old Street, London, E.C.1*

Two unfortunate errors in the interpretation of results have led to erroneous statements about the nutritive value of bread fortified with lysine.

Rosenberg & Rohdenburg (1952) showed that lysine fortification increased the protein efficiency ratio of bread from approximately 1.0 to 2.0 (and similar figures were obtained by Hutchinson, Moran & Pace (1956)). This was described as an increase of 70–100% but was interpreted by Jolliffe (1953) and Flodin (1953) and quoted by Horder, Dodds & Moran (1954) as 'can increase the availability of the protein for growth from 70–100%—a considerable increase in the biological value

of that protein'. This statement implies that the lysine-fortified bread protein has a biological value of 100.

This misinterpretation was confirmed by erroneous conclusions drawn from their own experiments by Rosenberg & Rohdenburg (1952) and by Rosenberg, Rohdenburg & Baldini (1954). These workers were unable to increase the P.E.R. of bread protein by the addition of any amino-acid other than lysine and concluded that the only amino-acid deficient in commercial bread was lysine.

The N.P.U. of the National Loaf (nominally 80% extraction) was examined by the carcass-nitrogen method (Miller & Bender, 1955) together with various supplements of amino-acids. The protein was fed at 9% and the diet was adequate in all other respects. N.P.U. of bread alone 46; + lysine (2.0%) 57; + lysine + threonine (1.4%) 74 (thus confirming the finding of Sure (1952) that threonine is the second limiting amino-acid); + lysine + threonine + methionine (1.1%) 80. The third limiting amino-acid in bread protein is thus methionine.

These values of 46 for bread and 57 for bread + lysine agree extremely well with the P.E.R.'s found by Rosenberg & Rohdenburg and Hutchinson *et al.* of 1.0 and 2.0 respectively (Block & Mitchell, 1946-7; Bender, 1956).

Thus bread even when supplemented by three amino-acids still has N.P.U. below 100 and the increase on supplementation with lysine, although twofold when measured as P.E.R., is only an increase from 46 to 57 when measured on a percentage scale.

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Flour and bread, prepared with or without treatment with chlorine dioxide, as long-term sources of vitamin E for rats. By T. MOORE, I. M. SHARMAN and R. J. WARD, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

The destruction of vitamin E in flour by ClO₂ was reported by Moran, Pace & McDermott (1953) and confirmed by ourselves (Moore, Sharman & Ward, 1957). This communication describes two further experiments, firstly on flour and secondly on bread, in which ClO₂, used at the ordinary commercial level as an improver, has again been shown to cause destruction of the vitamin. The experiments have taken the form of prolonged feeding trials.

Flour of 80% extraction rate, either untreated or treated with ClO₂, was made up into a diet of flour 85.5 parts, casein 5, lard 8, dried yeast 8 and salts 4. Supplements of vitamins A, D and K were provided. Female weanling albino rats, given

this diet made with untreated flour, developed no signs of vitamin E deficiency during a period of 509 days. In rats given ClO_2 -treated flour, however, vitamin E deficiency was indicated during life by haemolysis tests, dental depigmentation, subnormal body-weights, incipient paralysis and in some instances by skin lesions. At autopsy the uteruses of the deficient animals were found to be brown, the intra-peritoneal fat was sparse or absent, and the vitamin A reserves of the livers were lower than in the rats given untreated flour.

Bread was baked from the same flour with the addition of 0.3% calcium carbonate, and 1 oz. yeast was used in each 2 lb. loaf. It was given to young male albino rats, and was supplemented only with water and one drop of halibut-liver oil per rat weekly. The rats given bread made from untreated flour grew rather slowly to weights of 312–411 g, and after 441 days showed no evidence of vitamin E deficiency. Those given bread from flour treated with ClO_2 reached only slightly lower body-weights, of 273–386 g, but became deficient in vitamin E as judged by haemolysis tests, dental depigmentation, and degeneration of the testes. Enough vitamin E survived the baking process, therefore, to prevent avitaminosis E in the rat when untreated flour was used, but not when ClO_2 was used as an improver.

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The fluid intake of children, adults and the elderly. By E. R. BRANSBY,
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An enumeration of the 'cravings' of some pregnant women. By J. M. HARRIES and T. F. HUGHES (introduced by D. F. HOLLINGSWORTH), *Scientific Adviser's Division (Food), Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1*

The 'cravings' of pregnant women for various foods and even for inedible substances are well known, but there is disagreement in the literature about the significance of this phenomenon, which is of interest to the student of food habits. Nowhere is there any factual information. In 1936, a B.B.C. broadcast in the series 'Is there a Doctor in the House?', was devoted to pregnancy; these desires were mentioned, and listeners were invited to write to the B.B.C. about them. Of the 514 letters received 509, reporting 820 pregnancies, have been classified and are described by courtesy of the B.B.C. because they provide a unique series of data. They cannot be considered representative because of the possibilities of bias on the side of the unusual and by what was said in the broadcast.

Of the 991 'cravings' reported, 261 were for fruit, 105 for vegetables, and 187 for substances normally considered inedible. Of the cravings for fruit, most were for those readily available such as apples (76) oranges, (68) tomatoes (31) and

lemons (16). Of the 105 cravings for vegetables, 72 specified that these had to be raw. Other foods mentioned often were confectionery (79), pickled foods (66) and cereal products (65, of which 48 specified that these had to be in the dry raw state). There were 17 instances of a craving for spices and condiments eaten neat. Of the cravings for inedible substances, 35 were for coal and coal dust, 17 for soap, 15 for disinfectant, and 14 for toothpaste. The supposedly popular wall plaster was mentioned once, whitewash twice, and chalk four times. There were 193 instances of aversions during pregnancy from substances normally liked, 78 of them for tea, 24 for tobacco (smoking) and 22 for coffee.

There were also certain features common to many letters: many correspondents stressed the seriousness of these 'cravings' during pregnancy and the lengths to which they went to satisfy them, though later they themselves treated them with amusement; many mentioned the sense of secrecy they experienced and how they kept their 'cravings' secret even from their husbands; also it seems that they are usually experienced early in pregnancy.

The seventy-six reported instances of 'cravings' for two or more substances during the same pregnancy exhibit no pattern either of similar or contrasted substances being craved at the same time. The fifty-seven instances of the same substance being craved in each of several pregnancies by the same person also seem to be randomly distributed within the main pattern.

A rachitogenic factor in pig's liver. By M. E. COATES and G. F. HARRISON,
National Institute for Research in Dairying, Shinfield, near Reading

Yeast, green cereals and hay have been shown to have rachitogenic activity (Braude, Kon & White, 1943; Ewer & Bartrum, 1948; Grant, 1951; Weits, 1952; Raoul, Marney, Le Boulch, Prelot, Guerillot-Vinet, Bazier & Baron, 1957).

A rachitogenic factor in pig liver was first observed in the course of an attempted assay of vitamin D₃ by the method of Olsson (1941). A group of eighteen chicks receiving no vitamin D₃ had a mean tarso-metatarsal distance (T.M.T.) of 2.70 mm; a similar group receiving 10% raw liver in the diet had a mean T.M.T. of 3.07 mm.

Exp. no.	No. of birds/ group	Mean T.M.T. (mm) after addition (equivalent to 20% raw liver) to basal diet of:						
		No addition	Solvent extraction		Aqueous extraction		Dried cooked liver	Liver ash
			Extract	Residue	Extract	Residue		
1	18	3.21	3.60	4.10*				
2	18	2.87	2.32	3.22				
	15	2.79	2.85	3.50**				
4	15	3.22	3.50	3.79	3.42 (oven-dried)	3.31	3.12	3.30
5 (pooled)	45	2.46	—	—	2.78*** (freeze-dried)	2.61		

* $P < 0.05$. ** $P = 0.02$. *** $P < 0.01$. P = probability that the difference from the control group would arise by chance in a homogeneous population.

Subsequent experiments showed that extraction with organic solvents removed some of the rachitogenic activity together with any vitamin D₃ present in the liver.

Most of the rachitogenic activity remained in the residue. Extraction of this residue with water removed most of the activity, which was destroyed when the aqueous extract was dried in a hot-air oven, but remained on freeze-drying.

Neither dried cooked liver nor liver ash was active. The findings are summarized in the table.

Recent experiments indicate that the equivalent of 20 g raw liver counteracts the antirachitic activity of about 3 i.u. vitamin D₃.

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Nutritional fibrosis of the liver. By K. O. GODWIN, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Fibrosis of the liver of experimental animals may be a sequel to two types of injury associated with nutritional imbalance: (i) acute massive necrosis due to either deficiency of sulphhydryl groups or of α -tocopherol, or of both and (ii) fatty infiltration resulting from a shortage of methyl groups.

Although the liver lesion, progressive fibrosis, found in the human subject suffering from protein malnutrition more nearly resembles that found in (ii) than in (i), it is doubtful whether it can be explained on the basis of results of the experimental work on either type.

Davies (1952), in reviewing the liver changes in kwashiorkor, notes the initial localization of fat in the periphery of the lobule which may be followed by infiltration of the portal triads by round cells and a fine fibrosis at the periphery of the lobule which then extends in a stellate fashion. A similar description of the histology of the lesion is given by Walters & Waterlow (1954) who postulate a dual aetiology—malnutrition and malaria; they contend that malnutrition may cause damage to the liver 'if that tissue has already been made irritable and hyper-reactive by some other stimulus, such as malaria'. Macdonald & Thomas (1956) offer evidence from experiments on rabbits in support of a similar hypothesis. Platt (1956) holds the view that whilst malnutrition and malaria both play a part in the development of liver fibrosis in Gambian children, the role of the infection is to contribute to the degree of malnutrition. There has, however, as yet, been no direct experimental evidence of the production by dietary deficiency of lesions similar to those found in human subjects in protein malnutrition.

In the course of the examination of a large number of rats fed on low-protein diets, some for 2 or more years, it has rarely been possible to induce anything more

than a mild diffuse fibrosis of the liver, in spite of the invariable occurrence of fatty infiltration of the liver.

Evidence has now been obtained that pigs maintained on low-protein diets from an early age (2–5 weeks) for periods of from 3 to 18 months and showing no signs of infection, develop liver lesions having the features of those found in protein malnutrition in the human subject. The pig livers show periportal fatty infiltration and fibrosis which is most marked in those animals which have been the longest time on a low-protein diet.

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Chronic protein deficiency and some congenital abnormalities of the eye of the rat. By D. S. McLAREN, *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

Congenital abnormalities of many kinds, including those of the eye, have been attributed to numerous maternal nutritional deficiencies, but there is no mention in the literature of such defects having been produced by a diet deficient in protein. It is generally stated that in the rat a dietary level of protein below 5% is insufficient for ovulation and reproduction. This communication describes for the first time congenital defects attributable to a chronic deficiency of protein.

Female rats were made chronically protein-deficient by being fed a diet from weaning containing less than 4% protein. Only one successful mating has been achieved with a stock male fed on the low-protein diet for a few days before mating. This resulted in a litter of four which it was impossible to rear, as the mother had started to eat them.

No external abnormalities were evident but sections of the eyes showed them all to be smaller than normal. The most marked changes were in the lens, which showed almost complete absence of normal fibre formation and the epithelium consisted of a disorderly mass of irregular cells extending far into the anterior part of the lens. The lens bow was barely recognizable. There was pronounced reduction in the intensity of staining of the lens by Bennett's method for sulphhydryl groups. Amongst other changes was consistent absence of strongly periodic acid—Schiff (PAS) positive-reacting material normally found at birth in the zone of separation of the lids.

The eyes of all twelve pups in two subsequent litters from the same pair of rats, after they had been put on to stock diet, showed at birth improvement in orderliness of lens-fibre formation, regularity of the epithelium and lens bow and an increase

in intensity of staining by Bennett's method. However, there was globular degeneration of some lens fibres with interfibrillar clefts and much subcapsular debris. The PAS-positive material between the lids was consistently present.

A fourth litter of four has been reared by a stock rat, the experimental mother having failed to lactate, and the eyes of all show clinically no abnormality.

The effects of low-protein diet and of methionine sulphoximine on the sulphhydryl content of the lens of the eyes of the rat. By K. BAGCHI (introduced by B. S. PLATT), *Human Nutrition Research Unit, Nutrition Building, National Institute for Medical Research, The Ridgeway, Mill Hill, London, N.W.7*

The sulphhydryl content of the lens is much higher than that of any other tissue. In most forms of cataract and precataractous conditions, sulphhydryl compounds (glutathione, protein-bound SH and SH-containing enzymes) are decreased in amount and activity; their presence in the lens is believed to be important for the maintenance of transparency.

Whilst there is evidence that there is a relationship between the concentration of SH-containing substances in the tissues of animals and the amounts of the sulphur-containing amino-acids in their diet, there is no published work on the effect of differences in the quantity of these nutrients in the diet and the -SH content of the lens.

In animals on an adequate protein diet (18%), the SH content of the lens, determined by a colorimetric method using Bennett's reagent (Flesch & Kun, 1950), increases very rapidly from time of weaning (3 weeks) and reaches a maximum of 250-300 mg/100 g fresh lens at the age of 150-200 days. Thereafter it remains stationary or very slowly decreases with age. On low-protein diet (4%) the SH content remains at approximately the level found at weaning and the maximum value attained was about half of that of the control animals. However, at this low level no clinical abnormality was observed nor could any changes be detected on examination with the ophthalmoscope or slit-lamp microscope. Supplementation of the low-protein diet with methionine and cysteine brought the SH value to the level of that of the control group. Conversely, the lens SH of animals of the control group when kept on low-protein diet showed a rapid reduction until it reached about the same value as those in the experimental group.

By giving methionine sulphoximine, an antimetabolite of methionine, by intraperitoneal injection (25 mg/kg body-weight) to rats on 4% protein diet, the SH content of the lens was still further reduced and in 4-5 weeks the amount remaining was so small that it could not be estimated. Simultaneously with this reduction, lens opacity developed and ultimately a mature cataract formed. Histological examination of this cataract revealed gross damage to lens fibres, with vacuolation and hydropic changes, and also to the epithelium, especially at the equatorial bow. Early lens changes were reversed by supplementation of the diet with methionine.

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Further studies on the effects of thiamine deficiency and of caloric deficiency on behaviour in the rat. By MELEK KHAIRY and R. W. RUSSELL, *Department of Psychology, University College, London*, and JOHN YUDKIN, *Department of Nutrition, Queen Elizabeth College, University of London*

With Knopfmacher, we have previously reported that thiamine deficiency did not affect performance of rats in behaviour under stress or in rate of learning (Knopfmacher, Khairy, Russell & Yudkin, 1955, 1956). On the other hand, pair-fed control rats, with adequate thiamine but a reduced caloric intake, were more active, showed more trial and error, and learned more rapidly than the general control rats with adequate thiamine and free access to food.

With three similar groups of rats, we have now compared (1) conditioning to one avoidance situation, (2) the behaviour in a conflict situation and (3) conditioning to a second avoidance situation. In the first, each animal was placed in a cage and 20 sec later a platform inserted. After a further 10 sec, the floor of the cage was electrified. The conditioning occurred when the animal jumped on to the platform in the 10 sec before the shock was given. In the second situation, the procedure was modified by electrifying both floor and platform, so that the shock could not be avoided by jumping on to the platform. In the third, a lever was inserted instead of the platform. The shock could now be avoided again if the animals learned to press the lever within the 10 sec before the floor was electrified.

The results suggest that thiamine deficiency in itself did not affect conditioning. It did however profoundly affect behaviour in the conflict situation. The thiamine-deficient animals showed greater perseveration of the first conditioning, that is they persisted in jumping on to the platform even though by so doing they did not avoid shock. They showed shorter latency, in that they jumped sooner after the stimulus of inserting the platform. They showed greater oscillation, jumping more frequently on and off the platform. Finally, they showed much greater displacement activity, as indicated by frequently biting the cage.

The animals with caloric deficiency conditioned with fewer trials and with a shorter latency of response. In the conflict situation, they tended during the first trials to show the same perseveration and latency as the thiamine-deficient animals, but later there was a decline in perseveration and an increase in latency. In one measure of behaviour, the amount of oscillation, they were throughout the trials significantly different from the general control group. This is consistent with our previous finding that caloric deficiency results in greater activity and more trial and error.

These experiments will be reported in more detail in the *Quarterly Journal of Experimental Psychology*.

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The effect of pigeon-crop milk and dried egg on growth in chicks. By
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Pace, Landolt & Mussehl (1952) attributed to a vitamin the marked growth response they obtained in chicks by supplementing a basal corn—soya diet with 5 g pigeon-crop milk/bird/day and there is little doubt that their basal diet, which contained no animal protein, was deficient in vitamin B₁₂ and possibly in other B-group vitamins.

The work of Pace *et al.* (1952) has been repeated and the effect of supplementing a good chick starter ration with crop milk has also been studied. Shortage of crop milk limited the number of chicks to 5/treatment. A significant growth response due to crop milk ($P < 0.01\%$) was obtained during the 2nd week of the experiment with both basal diets. The starter ration contained 7% white-fish meal, 6% dried skim milk, 2% dried yeast, 2% dried lucerne, and 1% Drivite (a vitamin A and D supplement), so it would seem probable that the growth-promoting effect of the crop milk with this ration was due not to the vitamins, but to the fat and protein it contained. (On a dry matter basis the crop milk contained 36% ether extract and 62% crude protein). The influence of an alternative source of supplementary fat and protein, namely dried whole egg, on chick growth was therefore studied. The treatments (ten chicks/treatment) were: E, control; F, control + 10% egg; G, control + 40% egg; H, control + 40% egg on day 1, 35% on day 2, the egg being reduced by 5% each day so that 10% was fed on day 7. From the 8th day all chicks were fed the control ration (the Shinfield chick mash of Coates, Davies & Kon, 1955). The chicks on all the egg-supplemented diets gained at a significantly ($P < 0.01\%$) greater rate than the controls during the first 7 days, the mean weekly live-weight gains being 41.0, 48.6, 49.0, 49.2 g for treatments E, F, G, H respectively. During the subsequent 4 weeks there were no significant differences between treatments.

These results suggest that the growth-promoting effect of the whole egg was due to the gross nutrients it supplied, and the growth-promoting effect of egg yolk on chicks observed by Denton, Lillie & Sizemore (1954), Arscott (1956) and Hopper, Scott & Johnson (1956), attributed in the last two papers to an unidentified growth factor, may also have been due to the fat and protein in the egg.

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The effect of implanting diethyl-stilboestrol and hexoestrol on the growth of fattening sheep and cattle. By G. E. LAMMING and A. J. W. BROOME, *University of Nottingham—School of Agriculture*

It is now well established that the subcutaneous implantation of 12–15 mg stilboestrol in sheep and 30–120 mg stilboestrol or hexoestrol in cattle can produce a considerable increase in the growth rate of these animal fattening under English conditions (Aitken & Critchon, 1956; Burgess & Lamming, 1957; Lamming, 1957). It appears that hexoestrol may have certain advantages over stilboestrol since it is claimed to be less active orally for the human (Bishop, Kennedy & Wynn-Williams, 1948). This report is concerned with comparisons between stilboestrol and hexoestrol as growth promoters when administered at various dosage levels with and without excipient.

In the sheep experiments a total of 384 sheep were used from four different flocks. The animals of each flock were subdivided into the required number of groups on a live-weight basis before treatment. They were weighed at intervals throughout the test period and, when the animals of any group reached slaughter condition, the corresponding animals (on an initial live-weight basis) from the remaining groups were drawn out so that all could be slaughtered together. In this way direct comparisons on the effect of treatment were possible. The results are summarized in the table.

Effect of implanting hexoestrol and stilboestrol into fattening sheep

	Treatment	No./group	Mean Initial weight (lb.)	Mean gain/day (lb.)	Increased gain/day (%)	Killing out percentage
A 157 Halfbred × Suffolk males, 82 days duration	C	32	87.5	0.217	—	51.0
	12S	34	87.7	0.276	27.2	50.7
	12H	27	87.1	0.302	39.2	50.5
	10S + 5E	31	86.1	0.254	17.0	50.8
	10H + 5E	33	89.9	0.262	20.7	50.8
B 106 Oxford males, 165 days duration	C	40	82.1	0.188	—	53.0
	12S	40	83.8	0.232	23.4	51.6
	15H	26	84.3	0.257	36.7	53.8
C 50 Oxford × Half-bred males, 165 days duration	C	15	74.5	0.186	—	
	12S	15	73.9	0.248	33.3	
	15H	20	78.4	0.218	17.2	
D 66 Oxford × Halfbred males, 39 days duration	C	11	94.9	0.60		
	12S	18	95.8	0.70	16.6	
	10S	13	91.6	0.67	11.6	
	10S + 5E	12	95.6	0.76	26.6	
	5S + 5E	12	90.7	0.67	11.6	

C = Control; S = mg stilboestrol; H = mg hexoestrol; E = mg excipient

The growth-promoting effect of hexoestrol appears to be equal to that of stilboestrol, both treatments significantly increasing average daily gain with no significant effect on killing out percentage. Implantation with 12 or 15 mg tablets appears to produce a greater response than 10 mg or less. The addition of 50% excipient reduces the initial response and the total extra gain is therefore reduced.

With Friesian steers on pasture during the summer of 1956 implantation with 36 mg stilboestrol or 30 mg hexoestrol produced increases in average daily gain of 53% and 46% respectively (Lamming, 1957). In September 1956, ten of a group of twenty Shorthorn steers and eleven of a group of twenty-one Shorthorn heifers grazing pasture were implanted with 36 mg stilboestrol, the remainder acting as controls. The treated males gained 55% faster over a test period of 67 days (2.6 lb. compared with 1.7 lb. per day) and the treated heifers gained 15½% faster (2.1 lb. compared to 1.8 lb. per day) over a similar period. Treated females had normal oestrous cycles of similar length to controls and showed normal oestrus behaviour.

With cattle in yards similar increases in average daily gain due to implanting stilboestrol have been obtained.

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