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ABSTRACTS OF COMMUNICATIONS

The Eighty-fifth Meeting of The Nutrition Society (Thirty-ninth of the Scottish Group) was held at the Golden Lion Hotel, Stirling, on Saturday, 27 February 1954, *at 2 p.m., when the following papers were read:*

The Retention of Vitamin B_{12} by the Chick Receiving All-vegetable Rations.

By D. H. SHRIMPTON, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Chicks from a vitamin B_{12}-depleted breeding flock were reared on the all-vegetable diet A containing: groundnut meal 35%, yellow-maize meal 22%, wheat, oats, miller's offals, grass meal, brewer's yeast, minerals and cod-liver oil. Parallel groups were tested with diet B in which soya-bean meal replaced the groundnut meal—there is evidence that the inclusion of soya-bean meal in a ration raises the chick's requirement for vitamin B_{12} (Bird, Rubin & Groschke, 1948; Carpenter & Duckworth, 1951).

The vitamin B_{12} content of chicks aged 3 days and 28 days was determined with *Lactobacillus leichmannii*, using cyanide extraction. In 25 days, the vitamin B_{12} content increased by 380% (see Table 1), although neither ration contained any vitamin B_{12}, and the chicks were housed on wire screens to prevent coprophagy. (Work in progress on the origins of the exogenous source of vitamin B_{12} retained by these birds will be reported later.)

Age of chicks (days)	3		28					
Supplement	None		None		7 μg vitamin B_{12} by mouth		7 μg vitamin B_{12} by injection	
Diet	Grain	Grain	A	B	A	B	A	B
Live weight of chick (g)	40*†	40‡	170‡	153‡	220‡	232‡	225‡	242‡
Total vitamin B_{12} per chick (μg)	0·28†	0·13	0·49	0·50	0·9	1·1	3·1	2·8
Distribution of vitamin B_{12} in tissues								
Residual carcass§ (mμg/g fresh weight)	7·5†	3·8	2·3	2·7	3·3	3·5	9·5	5·6
Liver (mμg/g fresh weight)	38†	1·6	19	16	51	45	175	143
Gut (mμg/g fresh weight)	14†	6·5	5·6	6·2	7·5	12	16	15

* Mean of five chicks.

† Chicks from non-depleted breeding birds.

‡ Mean of twenty chicks.

§ After the feet and skin are discarded and the liver and gut removed, the remainder is termed 'residual carcass'.

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The retention of known doses of vitamin B_{12} by these chicks was studied in further groups from the same hatches. Seven doses of 1 μg 'cytamen' vitamin B_{12} were given orally by pipette or intramuscularly by injection during the 28 days of the experiment. In these chicks, the total content of vitamin B_{12}, and also its distribution, is shown in the table together with their mean weights. Intramuscularly administered vitamin B_{12} was more efficiently retained than the oral doses. This is in line with a report that orally administered vitamin B_{12} is only 50% as effective in promoting growth as equivalent intramuscular dosage (Stokstad, Jukes, Pierce, Page & Franklin, 1949).

No significant difference was observed in the retention of vitamin B_{12} in chicks fed diet A or diet B, or in its distribution in the tissues.

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The Origin of Tetany in Magnesium Deficiency. By K. L. BLAXTER and J. A. F. ROOK, *Hannah Dairy Research Institute, Kirkhill, Ayr*, and A. M. MACDONALD, *Royal Hospital for Sick Children, Glasgow*

An experimental magnesium deficiency can be produced in calves by feeding an artificial diet from which magnesium is excluded (Blaxter & Rook, 1953; Thomas & Okamoto, 1953). This syndrome is reversed and prevented by magnesium, appears to be uncomplicated by other nutrient deficiencies, and is characterized by hyperirritability, tetany and terminal convulsions. Evidence now accumulated suggests that the tetany and hyperirritability are associated with abnormalities of neuro-muscular transmission: they are not due to (a) central nervous damage, or (b) failure of magnesium-activated enzyme systems in muscle cells, and/or the toxic accumulation of metabolites normally removed in such systems. The evidence against (a) is firstly, that the syndrome could be reversed suggesting no neural cell degeneration, and secondly, that no biochemical, pathological or histological change has yet been found in the C.N.S. or peripheral nerves of calves dying in convulsions. The magnesium content of the brain was normal. Evidence against (b) is firstly, that the magnesium concentration in soft tissues was not reduced, secondly, that no reduction of the phosphatase activity was observed, and thirdly, that abnormalities of carbohydrate metabolism such as were produced in thiamine-deficient calves did not occur. The small increases in urinary pyruvate and blood lactate and pyruvate in magnesium-deficient calves was associated with the large increase in muscular activity during tetany and the hyperirritable phase. Comparable muscular activity in normal calves as judged by responses of body temperature and of pulse rate gave comparable changes in pyruvate metabolism. Neuromuscular transmission is thought to be involved since the deficient animals were

very sensitive to anti-cholinesterase drugs. Furthermore, the greatest relative depletion of magnesium occurred in the extracellular fluids of the body. In view of the observations of Del Castillo & Engbaek (1953) it seems probable that magnesium deficiency either alters the end-plate potential at neuro-muscular junctions or alternatively considerably affects the activity of acetyl cholinesterase or choline acetylase.

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Anaemigenic and Lipogenic Actions of Dietary Calcium Carbonate.

By W. A. GREIG, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Feeding a 2% supplement of calcium carbonate to mated pairs of albino mice on the 'Sherman B' diet induced a microcytic hypochromic anaemia in both the dams and their litters, and caused severe fatty infiltration of the liver in the young at weaning (Greig, 1952). Increasing the iron content of the diet from 35 to 45 p.p.m. largely offset these effects, and also raised the haemoglobin level of the unsupplemented group. Arising from these findings is the question of whether the effects of the calcium carbonate ($CaCO_3$) and iron supplements were independent or related.

Although the individual effects of both $CaCO_3$ and iron supplements were strong, and opposite in their directions, there was no apparent interaction between them. This might suggest that the effects of the two supplements were not dependent upon one another, and, therefore, that the anaemia caused by the $CaCO_3$ was not the result of an induced deficiency of iron.

However, iron absorption is known to be more efficient in severely-deficient than in mildly-deficient or normal animals, so that if the $CaCO_3$ supplement had indeed interfered in some way with iron availability, this effect might have been balanced by an increased efficiency in iron absorption. This could explain the failure to detect an interaction, and thus leave the question still open.

Experiments were therefore carried out to determine whether the anaemia and the other abnormalities might have resulted from some other deficiency induced by $CaCO_3$. Substances which might affect haemoglobin synthesis (e.g. copper, pyridoxin), and liver fat (choline, inositol, thyroid extract) were fed in excess in the presence of $CaCO_3$, but all failed to prevent the appearance of lesions. A third possibility—that $CaCO_3$ exerts a direct depressant effect on haemoglobin formation —was discounted by the finding that, when a diet very rich in iron was fed, $CaCO_3$ caused only a slight reduction in haemoglobin concentration unless administered at a very high level.

It is concluded that $CaCO_3$ exerts its anaemigenic action through some interference with the assimilation or utilization of iron. No direct cause for the lipogenic action of the supplement has been discovered; it is probably an indirect effect, secondary to the anaemia, as a result of inadequate oxidation of normal preformed fat.

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Artificial Milk Mixtures Prepared by the Mothers of Young Babies.

By F. E. HYTTEN, *Department of Midwifery, University of Aberdeen*

A recent inquiry into some aspects of infant nutrition, showed that many infants fed on officially recommended artificial milk mixtures were making poor progress, suggestive of underfeeding.

Feeds were made up in the laboratory according to the printed instructions, from both partly-skimmed (P.S.M.) and full cream (F.C.M.) National Dried Milks (N.D.M.). The milk powder was first scooped up loosely and levelled off, then replicated with tight packing of the measure. Analyses of these feeds indicated a range of energy intake from 62 Cal./kg/day for a 10 lb. (4·5 kg) baby on normal measures of P.S.M. to 119 Cal./kg/day for an $8\frac{1}{2}$ lb. (3·9 kg) baby on packed measures of F.C.M. The average infant requires about 120 Cal./kg./day (Smith, 1951); by this standard, P.S.M. makes a feed deficient in energy value, and F.C.M. gives adequate values only with tight packing of the measure.

The M.O.H., Aberdeen, arranged for Health Visitors to sample feeds, prepared before their arrival, in Aberdeen homes, for babies under 3 months old, and note the total volume of the feed and the number of feeds given daily. Of 100 samples, seventy-three were made from N.D.M. (about half from P.S.M.), twenty-three from dilutions of cow's whole milk, and four from proprietary dried milks. Extra carbohydrate was added mostly as ordinary cane-sugar or brown sugar, but in two cases as glucose.

Analyses showed compositions (in g/100 ml.) ranging for fat from 0·80 to 5·30 (mode 1·0–1·5), for protein from 1·54 to 5·41 (mode 2·0–3·0), for lactose from 1·78 to 8·02 (mode 3·0–4·0) and for sucrose from 0·21 to 13·20 (mode 4·0–5·0). The proportion of total calories derived from carbohydrate ranged from 31 to 78% (mode 50–60).

Calorie intakes/kg body-weight/day were calculated for each infant from the reputed total daily intake and the expected weight of the baby (Paterson & Smith, 1947). The modal value lay between 90 and 100 Cal./kg/day and the range between 58 and 212.

The effects of this wide range of calorie intake have not been studied in detail but the general impression is that most babies having low calorie intakes are not thriving, and those with very high calorie intakes are suffering no ill-effects. There

is general agreement that it is difficult to overfeed a baby, at least on a reasonably well-balanced mixture. The effects of underfeeding are more important, and apparently common. Printed instructions on tins of N.D.M. (particularly P.S.M.) give feeds of low energy value in relation to modern ideas of infant requirements. Of 100 feeds prepared by Aberdeen mothers, seventy-eight appeared to provide less energy than was theoretically required.

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Studies on the Fate of the Major Plant Pigments in the Alimentary Tract of the Sheep and their Relation to the 'Chromogen' Marker Method for Determining Nutrient Digestibilities. By J. DAVIDSON, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Studies of the digestibility of nutrients are accelerated if ratio methods using indigestible markers replace time-consuming collection techniques. Dietary constituent markers such as lignin and indigestible protein have been used but have been widely criticized. Consequently, when Reid, Woolfolk, Richards, Kaufmann, Loosli, Turk, Miller & Blaser (1950) described a new ratio method based on extraction and measurement at 406 mμ of an 'indigestible' plant chromogen(s), considerable interest was aroused. The present study was made to identify and measure the major fat-soluble pigments comprising the chromogen(s) extracted from suitable dried herbage and corresponding rumen contents and faeces of sheep. Appropriate techniques were devised for extracting, separating and estimating these major pigments which comprise chlorophyll a, chlorophyll b, phaeophytin a, phaeophytin b, carotene and xanthophyll.

Digestibilities obtained by the chromogen method were compared with those obtained by the normal collection method, and a comparison made of the amounts of individual pigments entering and leaving the alimentary tract.

Digestibilities of dry matter by the chromogen method at 406 mμ were lower by 3 - 7% than those by the collection method. Variable degradation of all pigments took place in the alimentary tract and even under the highly buffered conditions in the rumen, chlorophyll was degraded. There would be little advantage in using any one of the plant pigments as a marker.

Wave-lengths between 400 and 420 mμ, in addition to 406 mμ, were used in the present study. Calculations based on measurements at 416 mμ gave digestibilities in reasonable agreement with those by the collection method. The 'most suitable' wave-length at which to take measurements varied from sheep to sheep on the same diet.

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It would be inadvisable to apply the 'chromogen' method extensively until comparative digestibility trials, feeding the roughage in question to the species being studied, have shown that at some wave-length between 400 and 420 mμ substantial agreement is given with reliable collection methods.

Dietary pigments were found in all rumen sediments whether separated at high or low centrifugal speeds. These pigments were identified by spectrophotometry as plant and not bacterial in origin, indicating contamination of all sediments with plant material. Work on bacterial preparations which have been separated by filtering and centrifuging techniques alone should be reviewed in the light of possible contamination with minute fragments of plant material.

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The Relationship between the Chemical Composition of Hays and their Content of Gross Digestible Energy. By D. M. WALKER, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Roughages such as hays, silages and straws are potential supplementary foods under winter conditions on hill farms. The determination of the relationship between the chemical composition of roughages and their content of gross digestible energy (G.D.E.) is a first step in assessing their nutritive value.

Opinions differ as to which scheme of chemical analysis is most useful for predicting the nutritive values of roughages. Schneider, Lucas, Cipolloni & Pavlech (1952) and Mitchell (1942) consider that a more accurate prediction can only be made when an analysis such as that suggested by Ferguson (1948) is followed. Others consider that the present analysis for proximate nutrients gives a good indication of nutritive value when an equation such as that of Schneider *et al.* (1952) is used, and that digestion with sulphuric acid alone gives a reproducible 'crude-fibre' value which is related to digestibility (Hallsworth, 1950).

The chemical composition of twenty-four mixed grass-clover hays and their content of G.D.E. for sheep have been compared. The regression equations relating the variables (x) to the digestibility of energy (y) were:

$y = 84{\cdot}4 - 2{\cdot}66\ x_1$ (lignin—Ellis, Matrone & Maynard, 1946)
$y = 108{\cdot}2 - 1{\cdot}76\ x_2$ (true cellulose—Norman & Jenkins, 1933)
$y = 112{\cdot}0 - 1{\cdot}72\ x_3$ (true cellulose—Crampton & Maynard, 1938)
$y = 111{\cdot}9 - 1{\cdot}59\ x_4$ (crude fibre—Association of Official Agricultural Chemists, 1950)
$y = 106{\cdot}2 - 1{\cdot}01\ x_5$ ('crude fibre'—5% H_2SO_4 digestion for 1 h, Hallsworth, 1950).

The actual G.D.E. values obtained from three readings were compared with the values predicted, and the limits of error noted which contained 50% and 95% of the observations.

Percentage of observations	Lignin (Ellis *et al.*)	True cellulose (Norman & Jenkins)	True cellulose (Crampton & Maynard)	Crude fibre (A.O.A.C.)	Crude fibre (5% H_2SO_4)
50	± 1·6	± 2·0	± 2·1	± 1·6	± 1·7
95	± 4·8	± 6·0	± 6·4	± 5·0	± 5·0

Crude-protein content was not related to G.D.E. Prediction was not improved by analysing for lignin and cellulose. Simple digestion with 5% H_2SO_4 for 1 h gave a 'crude fibre' as closely related to G.D.E. as any of the other variables.

The starch equivalents of the hays calculated from the G.D.E. values were consistently lower than those calculated by conventional methods.

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Plane of Nutrition and Energy Utilization by Sheep. By K. L. Blaxter and N. McC. Graham, *Hannah Dairy Research Institute, Kirkhill, Ayr*

Over a 6-month period two adult sheep were fed dried grass at five and seven planes of nutrition respectively, food intakes ranging from slightly below to (in one case) over three times, the maintenance level. Using indirect calorimetry, daily heat-production figures and complete energy, carbon and nitrogen balances were obtained on each of the final 4 days of a 14-day period at each level. The fasting metabolism was also measured from the 48th to 96th hour of fast, following feeding at the maintenance level for a period of 14 days. At this time, stable values of heat production were obtained and the R.Q. was 0·700–0·705.

Utilization of dietary energy became progressively poorer as the nutritive plane was raised, with no apparent discontinuity in the relationship at energy equilibrium. These results agree with others for cattle (Mitchell & Hamilton, 1932; Forbes, Braman & Kriss, 1928, 1930), rats (Forbes, Kriss & Miller, 1934) and rabbits (Hellberg, 1949). They do not agree with Marston's interpretation of the data from his sheep experiments (Marston, 1948) although recalculations by Hellberg do, in fact, reveal a curvilinear trend.

The starch equivalent, or net energy value of a food is thus not constant even for one productive process. In addition, calculation of the S.E. from the digestible nutrient and energy content of the material using Kellner's and other factors, gave figures widely divergent from those obtained calorimetrically.

Typical energy-balance data for one sheep are:

Intake (Cal./24 h)	Output (Cal./24 h) Faeces	Urine	Methane	Heat	Balance (Cal./24 h)	Body-weight (kg)
0	—	68	—	895	−963	43·6
1951	787	134	133	1250	−353	34·9
2891	1098	154	180	1392	+ 66	39·2
3841	1642	209	214	1641	+136	37·3
4723	1953	227	255	1843	+492	42·1
5804	2396	296	281	2085	+746	41·6
6791	2741	359	356	2432	+904	46·6
7605	3237	412	347	2626	+1100	49·7
Standard error of means	±23·4	±7·9	±5·6	±28·0	±35·1	——

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The Eighty-eighth Meeting of The Nutrition Society was held at the London Hospital Medical College, London, E.1, on Saturday, 22 May 1954, *at* 11 *a.m., when the following papers were read:*

Malnutrition and Hair Pigmentation. By B. S. PLATT and J. NAGCHAUDHURI, *Human Nutrition Research Unit, Medical Research Council Laboratories, Holly Hill, London, N.W.3*

Dyspigmentation of hair and skin is a feature of protein malnutrition in young children (Brock & Autret, 1952). We find reddish brown hair in malnourished infants in West Africa where the hair colour is normally jet black. Red hair is one of the signs pathognomonic of the form of protein malnutrition known by various names, including kwashiorkor (Williams, 1935).

Some melanin pigment of hair is extracted with dilute hydrochloric acid heated in a sealed glass tube at 120° (Arnow, 1938). The pigment (*B*) obtained by this method from black hair moves very slowly on filter paper (Whatman no. 1) in a mixture of equal volumes of ethylene chlorhydrin and 0·2 M-acetic acid; the pigment (*R*) from the red hair of malnourished subjects runs about 3 cm in 25 min at room temperature (*c.*22°). The presence of the pigment, which stains the paper slightly, is easily demonstrated as it fluoresces in ultraviolet light. These pigments (*B* and *R*) can be eluted from the paper; both have been shown to contain protein. The absorption spectra in ultraviolet light resemble that of tyrosine; in visible light (440 mμ–560 mμ) the spectra of *B* and *R* are like those of unoxidized and oxidized forms of melanin, respectively.

Pigment *B* is readily converted into one with the chromatographic and spectrographic characteristics of *R* by oxidation with hydrogen peroxide.

The pigment extracted from the black hair of hooded rats has properties similar to those of *B*; an extract from the pigmented hair of hooded rats on diets low in protein or low in methionine, or on normal diets but irradiated with ultraviolet light, behaves on chromatography like *R*.

There is evidence that keratin formation may be affected in protein malnutrition (Platt, 1953); tests for the distribution of –SH groups were therefore made with 1–(4–chloromercuriphenylazo)–2–naphthol (Bennett, 1951). There was a more intense and more extensive reaction in the skin of malnourished animals than in the normal ones. On the other hand, the skin of animals in which *R* was produced by irradiation had less than the normal sulphydryl reaction as judged by this method. The significance of this observation is not entirely clear. The cystine content of hydrolysates of hair yielding *R*, determined by the method of McFarren & Mills (1952), was found to be less than that of normal hair.

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Histochemical Changes in Scurvy. By C. RUTH HILL and G. H. BOURNE, *Histology Department, London Hospital Medical College, London, E.*1

Though the histological changes produced during the deficiency disease of scurvy have been known for many years, and have now been thoroughly investigated, the exact role which ascorbic acid plays in the metabolism of the cell is not yet known. Several authors have suggested that it is intimately associated with cellular syntheses and may be involved in the enzymatic mechanisms of the cell. It was thought that a histochemical study of the organs and tissues of scorbutic animals might serve to throw some light on this problem.

Pair-fed guinea-pigs between 200 and 250 g were fed on rat-cake supplemented with vitamins A and D, and in the case of the control guinea-pigs with 10 mg vitamin C daily. They were killed by a blow on the head after about 21 days and the various organs and tissues were treated with various histochemical reactions. Gomori's methods for esterase, lipase and acid phosphatase were carried out on acetone-fixed tissues. Apart from pancreas and liver, normal guinea-pig tissues contain very little esterase, but scorbutic pancreas gives a stronger reaction for esterase, and also for lipase. Scorbutic liver gives a stronger reaction for acid phosphatase, the hepatic cells near the central vein being more intense than those at the periphery of the lobules. Seligman's succinic-dehydrogenase method on fresh frozen sections revealed a marked decrease in activity of scorbutic liver, kidney and skeletal muscle, though there was no detectable decrease in cardiac muscle.

Bouin-fixed tissues were used for the periodic acid-Schiff (P.A.S.) test for polysaccharides, and formalin-lead-acetate fixed tissues were stained with toluidine blue for metachromasia (sulphuric esters). Hepatic cells of scorbutic liver show an increased basophilia with toluidine blue and Hassall's corpuscles in scorbutic thymus give a more intense P.A.S. positive reaction. Examination of costochondral junctions showed that the unorganized tissue characteristically seen in zones of ossification in scurvy, gives a strong P.A.S. positive reaction and in some cases so did the edges of the calcified cartilage matrix. The most striking results were obtained with scorbutic spleen where there is a great increase in the number of phagocytic cells of the red pulp which contain intensely P.A.S. positive droplets and granules. These droplets also contain iron and appear slightly yellowish in colour in haematoxylin- and eosin-stained specimens.

The results described here are of a preliminary nature and, while they confirm that significant metabolic changes occur in scorbutic tissues, their exact significance cannot at the moment be assessed.

The Effect of the Vegetable Ration on Carotene and Vitamin A in the Blood of Chronic Hospital Patients. By Z. A. LEITNER, *Claybury Hospital, Woodford Bridge, Essex* and 52 *Welbeck Street, London, W.*1 and T. MOORE and I. M. SHARMAN, *Dunn Nutritional Laboratory, University of Cambridge* and *Medical Research Council*

In an earlier paper (Leitner, Moore & Sharman, 1952) we reported that carotenoids and vitamin A in the blood plasma of a large group of patients, of both sexes, at Claybury Hospital averaged 53 μg and 118 i.u./100 ml. respectively. For healthy private patients averages of 133 μg and 148 i.u. were found. Further studies have now been made on the importance of the dietary régime, as opposed to the patients' abnormalities, which were usually psychological, as a cause of the low values found at Claybury. Fifty patients were given daily 170 g of either carrots or spinach alternately besides their existing sources of carotene. The combined average for carotenoids in both sexes rose from 38 μg/100 ml. before the dietary additions to 86 μg and 114 μg after the additions had been made for 17 and 44 days. Vitamin A levels were 125, 156 and 155 i.u. The extra vegetables, therefore, produced levels for both carotenoids and vitamin A which were close to those previously found for private patients.

In ten patients, selected for high carotenoid values, readings were continued after the extra vegetables had been stopped. Carotenoids fell from 130 μg to 108 and 65 μg after 7 and 144 days respectively. For vitamin A the decline was delayed until after the first 7 days, with levels of 154, 162 and 113 i.u.

Chromatographic analyses were made on the carotenoids of eleven patients not given extra vegetables. Strongly adsorbed 'xanthophylls', averaging 28 μg/100 ml. exceeded the weakly adsorbed 'carotenes', averaging only 10 μg. In three patients given the extra vegetables, however, carotenes slightly exceeded xanthophylls, with averages of 58 and 44 μg. Dosing of two patients with carotene in oil increased only the carotene fraction; in two patients dosing with lutein dipalmitate caused slight increases in the xanthophyll fraction only.

In the previous paper we mentioned that the female patients at Claybury not only showed the usual slight superiority over males in their carotenoid levels, but also reversed the usual tendency in having a slightly higher average for vitamin A. In the present experiments the superiority of the women in both carotenoids and vitamin A was again found before the dietary changes. After extra vegetables had been given the female superiority in carotene persisted, but the males had now a slightly higher average for vitamin A.

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Hydrocephalus in Rabbits Associated with Maternal Vitamin A Deficiency. By G. E. LAMMING (introduced by G. H. BOURNE), *University of Nottingham, School of Agriculture, Sutton Bonington* and J. W. MILLEN and D. H. M. WOOLLAM, *Department of Anatomy, University of Cambridge*

Studies were undertaken to determine the effect of subclinical vitamin A deficiency in the dam on the growth of young rabbits. The dams were fed on a diet containing less than 0·1 μg carotene/g food for approximately 14–18 weeks before mating and received an oral supplement of vitamins E and D. Control dams received the above treatment plus vitamin A. The studies revealed that hydrocephalus in the young of vitamin A-deficient dams occurred in a high percentage of cases (twenty-six out of thirty-five young examined). The hydrocephalus was often accompanied by emaciation and head retraction and less frequently by convulsions and paralysis. These signs usually occurred 3–10 weeks after birth. There were no abnormalities in the young from the control dams.

Coronal section of the skulls with the brain *in situ* revealed gross dilatation of the cerebral hemispheres in affected animals. In most cases the optic nerves showed marked constriction at the region of the optic foramina. Laminectomy showed that the cerebellum had herniated through the foramen magnum, but the fourth ventricle appeared normal. There was no gross deformity of the foramen magnum or base of the skull but examination of the region of the cerebral aqueduct revealed marked stenosis of the aqueduct. Colloidal carbon injected into the lateral ventricles during life did not pass beyond this constriction.

The high incidence of these signs in young from deficient dams and their absence in young from control animals indicates a causal relationship between maternal vitamin A deficiency and the appearance of stenosis of the cerebral aqueduct in the offspring. In a preliminary report of these findings (Millen, Woollam & Lamming, 1953) evidence was presented indicating that the hydrocephalus was probably not due to a genetic factor as had been reported in mice (Grüneberg, 1943).

Preliminary examination of coronal sections of the skulls of foetuses obtained when vitamin A-deficient dams were autopsied 29 days *post coitum* revealed that hydrocephalus was present in thirty-four out of forty-three foetuses examined. Eleven out of twenty-seven young which were born dead or were aborted by vitamin A-deficient dams also had hydrocephalus. These foetuses await further detailed examination of the brain and its surrounding and associated structures.

These findings suggest that a lack of vitamin A in the dam can affect the development of the brain in the young prenatally as well as in the postnatal period. The relationship of these findings to the increase in cerebrospinal-fluid pressure common during vitamin A deficiency in calves requires further investigation. These

investigations so far suggest that an increased cerebrospinal-fluid pressure may produce stenosis of the cerebral aqueduct which in turn produces an obstructive hydrocephalus in young rabbits. That hydrocephalus can occur prenatally as well as in the postnatal period indicates a need for a re-examination of the view that the nervous abnormalities common during vitamin A deficiency in laboratory animals are due to either a cessation of bone growth (cf. Wolbach & Hegsted, 1952*a*,*b*) or to abnormal bone growth (cf. Mellanby, 1944).

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A Physiological Constant and its Nutritional Significance. By R. L. Worrall, 31 *Braeside Avenue, Sevenoaks, Kent*

The following mathematical investigation provides a new method of measuring basal metabolism, and presents a hitherto unknown relationship of the physiological constant, 0·00482.

0·00482 is the caloric value of 1 ml. oxygen, when the R.Q. has its average value of 0·82 for the postabsorptive state. That is to say, the ratio of the total basal metabolic rate to the total basal oxygen consumption, in ml., has the standard value, 0·00482.

The same number, 0·00482, has been found by the author to be the standard value of the ratio of the creatinine coefficient to the total basal metabolic rate, for a normal adult of average weight (W = 70 kg), calculated from the formula of Brody (1935) for the rate of excretion of creatinine nitrogen in mg/day, C.N. = $12{\cdot}7 \times W^{0{\cdot}896}$, and from the formula of Kleiber (1947) for the total B.M.R., in Cal./day, B.M.R. = $70 \times W^{0{\cdot}75}$.

Thus for a normal adult of standard average weight, whose total basal oxygen consumption in ml./day is $O_2T.$, there is the equation

$$\frac{\text{B.M.R.}}{O_2T.} = 0{\cdot}00482 = \frac{\text{C.N.}}{W \times \text{B.M.R.}}$$

$$O_2T. = \frac{\text{C.N.}}{W \times (0{\cdot}00482)^2} \quad \ldots\ldots\ldots \quad (1)$$

Now let $O_2P.$ represent ml. of oxygen consumed per day in basal protein breakdown, during the postabsorptive state. According to the usual formula, $O_2P.$ = N.P.N. × 5·94, where N.P.N. represents mg non-protein nitrogen excreted per day in the urine during the postabsorptive state. From this formula and from equation (1), the proportion of total basal oxygen consumption due to protein

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breakdown, for a normal adult of standard average weight, is given by the equation

$$\frac{O_2P.}{O_2T.} = \frac{W \times 5{\cdot}94 \times N.P.N. \times (0{\cdot}00482)^2}{C.N.} \quad . \quad . \quad . \quad . \quad (2)$$

Taking the accepted figure of 12% as the usual proportion of total absorbed oxygen consumed in protein breakdown, the standard value of the ratio, $O_2P. : O_2T.$, as given by equation (2), is 0·12.

For an adult of any weight, the general equation is:

$$\frac{O_2P.}{O_2T.} = \frac{W^{0{\cdot}146} \times N.P.N. \times 0{\cdot}00519}{C.N.}$$

For routine estimations of the proportion of total basal oxygen consumption which is due to protein breakdown, the value of the ratio, N.P.N. : C.N., can be determined sufficiently accurately from analysis of one specimen of urine, collected first thing in the morning, the last meal of the previous day having been a light lunch containing no significant amount of protein. The only other measurement required is that of body-weight.

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A Study of the Effects of Completely Vegetarian Diets on Human Subjects. By J. F. DE WIJN (introduced by H. E. MAGEE), W. F. DONATH and H. C. VAN DER MEULEN-VAN EYSBERGEN, *The Dutch Institute of Preventive Medicine, Leyden*

In spite of much work we still do not know whether it is possible to maintain full health and activity over prolonged periods with little or no intake of animal protein. Investigations in this field have been carried out in the Dutch Institute of Preventive Medicine (J. F. de W. and H. C. v. d. M-v. E.) and in the Biochemical Department (W.F.D.). Groups of vegetarians volunteered to cooperate.

(*A*) One group of thirteen subjects had consumed no animal food for periods ranging from 2 to 15 years previous to investigation (vegans). These subjects were seen in follow-up investigation.

(*B*) A second group of lacto-vegetarians volunteered to use diets free from animal protein for several months, ranging from 2 to 6 months. They served as a control group.

Dietary questionnaire revealed a low calorie intake as an average and appreciably low intakes of total protein, calcium and riboflavin. Excessive intakes of provitamin A, nicotinic acid, vitamin C and iron were noted.

The amino-acid composition of the proteins seemed to be normal.

Metabolic investigations of the vegans showed considerable underweight when compared with both group *B* controls and with controls on mixed diet. B.M.R. calculated per sq. m. body surface of long-standing vegans was significantly higher than B.M.R. in both control groups. Pulse rate, blood pressure and pulse pressure were higher in vegans than in controls. Vital-capacity rates were significantly lower for male subjects. In medical examination, symptoms which could be ascribed to malnutrition were recorded in minor degrees in nine out of sixty subjects. Almost all subjects were in good health and performed full employment in various jobs. Blood morphology showed a tendency towards macrocytosis in the majority of the subjects, three of whom had a previous history of macrocytic anaemia. The mean haemoglobin levels were not low. Vitamin B_{12} levels were normal.

Serum chemistry revealed no evidence of deficiency. The ratio albumin : globulin was significantly higher for long-standing vegans. Paper electrophoresis of serum protein showed high albumin fractions combined with low α_2-globulin and high γ-globulin fractions in four out of thirteen long-standing vegans. Non-protein rest and urea levels were normal. Excretion of uric acid and creatinine in 24 h urine samples were low.

The study revealed that the human organism often seems to have a remarkable power to adapt to various nutritive conditions and thus it may be possible for persons long accustomed to low protein intakes with no or little animal protein to exhibit lower requirements.

Protein Fractionation of Breast Milk of Nigerian Women. By O. BASSIR, *Area Pathology Laboratory, Westwood Hospital, Beverly, Yorkshire*

Some recent reports of researches carried out on malaria suggest that the widespread practice of breast feeding in West Africa is of even more importance to the general health of infants than has hitherto been conceived. Bruce-Chwatt (1954) has shown that the susceptibility of breast-fed rats to infection by *Plasmodium berghei* during the 1st month of life depends on the transmission of immunity from the nursing mother via milk. Hawking (1954) has confirmed Maegraith's observation (Maegraith, Deegan & Jones, 1952) that a diet of milk increases the resistance of rats to *P. berghei*, and gone further to show that this resistance may be lost by the addition of as little as 20 mg *p*-aminobenzoate/kg/day. Also, baby rats and monkeys were not infectable while being breast-fed. This suggests that the anti-malarial effect of milk feeds consists of at least two components—the PAB aspect and another.

One of Jelliffe's papers (Jelliffe, 1952) on the protein content of the milk of Yoruba women suggests that the concentration is not usually below normal. We have made an examination of the amino-acid composition of random samples of Yoruba mother's milk by the qualitative method of circular paper chromatography. Although the results have not been completely analysed, none of the

main groups of amino-acids normally present in milk seems obviously deficient in the forty cases studied. Methionine, valine, leucine, *iso*leucine, threonine, lysine, phenylalanine, arginine and histidine are always present.

As it appears reasonable to expect immune bodies to be transmitted in one or other of the protein fractions of milk, these fractions have been separated by paper electrophoresis in a barbitone buffer at pH 8·6. The figures obtained with milk from healthy English mothers breast feeding their babies show the three main components, lactalbumin, casein (two peaks) and a relatively small amount of lactoglobulin. Indications of the presence of other unidentified components can be seen (Heyndrickx & de Vleeschauwer, 1952). Those obtained with milk from Yoruba suckling mothers with healthy babies show similar peaks, but the globulin fractions in the younger specimens seem to be rather considerable. In underfeeding and failure to thrive of African children, electrophoretic patterns of milk were quite atypical. In marasmus, there are two or three fast-moving unidentified peaks, and practically no globulin. In gastro-enteritis there is a prominent peak of low mobility.

If the lack of uniformity—here demonstrated—in electrophoretic components of African milk has any nutritional significance, it is perhaps that the protein fractions they represent are the vehicle of transfer of preformed bodies that are necessary for encountering some of the natural hazards to which the tropical child is exposed.

In view of the difference, in manufacturing site, of gamma globulins and the other components of serum protein (Miller & Bale, 1954) liver disease and other disabilities in the suckling mother may badly influence the quality of the protein endowment of the milk, and thus limit the formation of antibodies and other defences of the body.

The samples of milk used in this investigation were obtained from Ibadan through the kind co-operation of Dr Davidson Nicol and Dr Tompkins.

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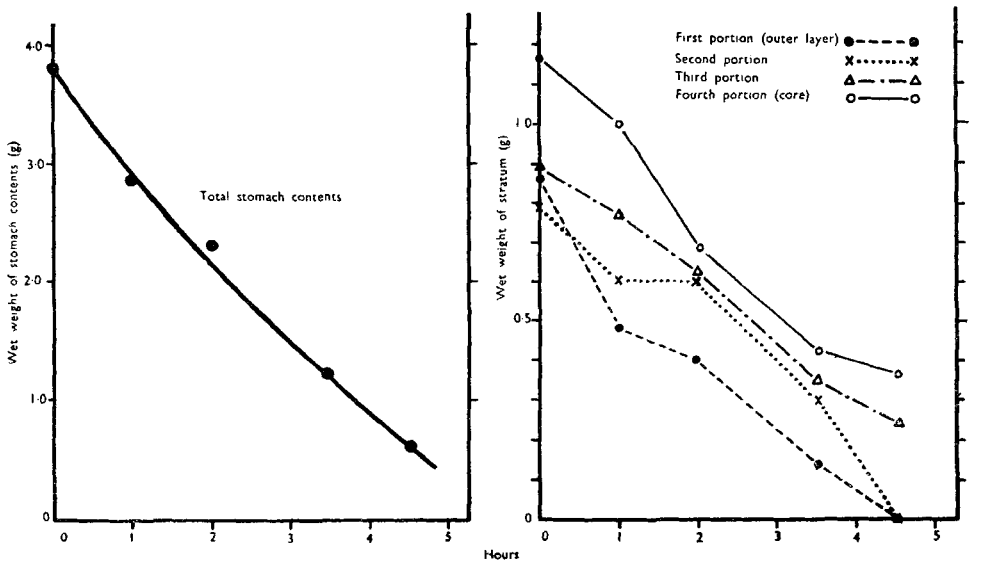
The Fate of Successive Portions of a Meal in the Rat's Stomach. By B. S. PLATT, *Human Nutrition Research Unit, Medical Research Council Laboratories, Holly Hill, London, N.W.*3

Stratification of food has been observed in the stomach of the horse (Colin, 1886), rat (Grützner, 1905), various domestic animals (Scheunert, 1931) and man (Cowgill, 1946).

On feeding successive portions of bread paste the first to enter the stomach constitutes the outer layer, the last eaten forming the core; during the 4 h after ingestion the portions were found to leave the stomach at the rates shown in the figure.



The quantities of four components of a meal remaining in the rat's stomach after various intervals. Five 6-week-old rats (stomachs empty and coprophagy prevented) were given consecutively four differently-coloured 1 g pellets of bread paste. Stomach contents were separated into single-coloured strata for weighing.

This observation is of some interest in relation to one feature of the mechanism of the supplementary action of proteins in view of the importance of supplying simultaneously to the tissues in the correct proportions the amino-acids they require for the synthesis of proteins (Geiger, 1950).

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The Behaviour of Milk in the Stomach of the Infant Rat. By B. S. Platt, *Human Nutrition Research Unit, Medical Research Council Laboratories, Holly Hill, London, N.W.*3

In the course of a study of the properties of rat's milk, samples were taken from the stomach content of suckling rats and it was observed that the milk clot could be separated into layers. The occurrence of strata of food in the stomachs of various

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animals (Platt, 1954) was recalled; however, when animals were suckled in succession by rats whose milk was tinted by previous injection with suitable dyes, the order in which layers occurred was found to be the converse of that when bread was fed, i.e. the outer layer was formed from the milk in the latest feed. More than one layer could be distinguished as the result of a single period of suckling. It seemed that the fresh milk flowed over the surface of the cheese-like clot already in the stomach; it then curdled and the whey separated.

Measurements of the water content of the outer shell of the clot and the inner core (excluding the portion of the stomach contents in the pyloric antrum which were undergoing digestion and were mushy) showed that the water content of the outer layer fell from about 70% initially to about that of the inner core (50%) in 30–40 min. At the same time the amount of lactose in the stomach decreased rapidly in the period when fluid was leaving the stomach (30–40 min). Assuming that rat's milk separates like cow's milk into curd and whey and that all the lactose is present in the milk serum (Van Slyke & Bosworth, 1916) then it might be inferred from the data obtained that nearly two-thirds of the whey left the stomach in 30–40 min after a meal. The remaining third was apparently contained in the interstices of the clot. The lactose in this fraction of the whey was, however, hydrolysed to glucose and galactose. When a new meal was taken the whey separating from it appeared to permeate the clot, displacing the whey remaining from the previous meal.

Approximately 0·07 g curd (50% moisture), equivalent to 0·12 ml. breast milk, left the stomach every hour. An infant rat's stomach with a content of 1·5 g would not empty for 20 h. This rate of emptying was confirmed by the observation, made many times, that curd from marked milk was present up to 20 h after ingestion. The curd left the stomach after digestion in the pyloric antrum in which regurgitated duodenal juices seemed to be involved.

Some of the breast milk entering a completely empty stomach soon left the stomach; the remainder formed a clot which contained pockets of air and mucus. It was many hours before the regular stratification of the meals appeared.

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Microbiological Assay of Protein Quality. 1. Growth of *Tetrahymena pyriformis* and Assessment of Response.

By W. R. FERNELL and G. D. ROSEN, *Research Department, J. Bibby & Sons Ltd, King Edward Street, Liverpool, 3*

A microbioassay of protein quality has been developed using the holotrichous protozoan *Tetrahymena pyriformis* W. This organism was selected because it

requires the same ten essential amino-acids as higher animals, it is able to digest both soluble and particulate protein, and it can be grown in pure culture on a chemically defined medium.

Studies on the growth of *T. pyriformis* on intact proteins have shown: (a) Highly aerobic conditions must be employed for maximum growth. This avoids acid production sufficient to cause death in rapidly growing cultures. (b) Ammonia is the end-product of protein metabolism. Unless sufficient glucose is included, protein is utilized as a primary energy source and the increased deamination can lead to conditions too alkaline for survival. (c) Under optimum conditions, populations up to $2{\cdot}5 \times 10^6$/ml. can be maintained alive and vigorous for 4–8 days in media containing up to 4% protein and 4% glucose. (d) The proteolytic system is sensitive to inhibitors such as occur in native egg albumen.

Growth response is measured by haemocytometric count. Turbidity, dry weight or protein-nitrogen content of the organisms cannot be used in the presence of food particles.

Criteria for protein quality based on weight gain or nitrogen balance cannot be applied to *Tetrahymena* until a quantitative separation of organisms from food can be achieved. The in vivo enzymic hydrolysis by the organism is an important feature of the assay but a direct comparison of protein quality by means of count cannot be made because growth depends upon the extent of proteolysis, which varies with the nature and physical state of the protein. It is assumed that, in the presence of glucose as an energy source, the ammonia produced is an index of the amount of protein utilized in the course of tissue synthesis, and that calculation of the number of organisms grown for a given ammonia production yields a measure of the efficiency of utilization of protein. As expected high and low quality proteins manifest high and low count : ammonia ratios respectively and nutritional values of proteins for *T. pyriformis* are therefore compared by means of this ratio.

Microbiological Assay of Protein Quality. 2. Comparison of Nutritive Values of Proteins. By G. D. Rosen and W. R. Fernell, *Research Department, J. Bibby & Sons Ltd, King Edward Street, Liverpool*, 3

The principle of the method of comparing the nutritive value of proteins has been outlined in the preceding communication (Fernell & Rosen, 1954). In practice the assay involves feeding protein at selected levels up to 60 mg protein nitrogen/10 ml. medium. The medium (based on that of Kidder and Dewey (Lwoff, 1951)) contains purines and pyrimidines, vitamins and minerals, and to this is added a solution or suspension of the test protein (pH 7·2). The mixture is autoclaved and sterile glucose is added aseptically. After inoculation and incubation at 25° for 5 days, populations and ammonia contents are determined. The response curves for count and ammonia against protein nitrogen fed are plotted for each protein. The area under the count curve is divided by the area under the ammonia curve

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for each protein and these ratios, when expressed relative to casein at 100, are referred to as relative nutritional values.

A preliminary survey of a range of animal and vegetable proteins has been carried out and a selection of results appears in Table 1, in which they are compared with net protein values for rats (Bender, 1953).

Table 1. *Relative nutritional values of proteins*

Protein	Nutritional values determined by *Tetrahymena pyriformis* W	Net protein values for rats (casein = 100)
Egg albumen	120–125	137
Wheat germ	110–120	112
Casein	100	100
Groundnut	48–54	—
Groundnut (overheated)	28–33	—
Gelatin	<5	0
Gelatin + tryptophan	37	—

These results are in reasonable agreement with those obtained by rat feeding. The beneficial effect of supplementing gelatin with tryptophan is quite marked and the deleterious effect of overheating groundnut protein is demonstrated.

The main advantages of the microbioassay are that it can be completed in 7–10 days, it requires only gram quantities of protein, and an in vivo rather than an in vitro protein hydrolysis is involved. Results so far indicate its potential value for the determination of safe limits for heat-processing of oilseeds during oil milling; it may also prove useful for the grading of fish and meat meals, which are of variable quality. It will be of interest to compare microbioassays of mixtures of animal and vegetable proteins used in compounded animal foods with the results obtained by conventional feeding trials on higher animals.

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The Nutritive Value of Six White Fish Meals of Known Origin. By K. J. CARPENTER, G. M. ELLINGER and D. H. SHRIMPTON, *Rowett Research Institute, Bucksburn, Aberdeenshire*

White fish meal is used in animal feeding as a standard source of high-quality protein and of some B-vitamins. However, the value can be lowered by severe processing methods (e.g. Maynard & Tunison, 1932; Bender, Miller & Tunnah, 1953).

For the present study meals were prepared under factory conditions with two different types of fresh filleter's offal and with three processes representing the range of British practice: *A* (initial drying in a batch agitated steam drier, 3 h at

250–300°F, then passage through a direct flame-drier), *B* (using a continuous agitated steam drier, with a countercurrent of ventilating air, 2½ h at 130–200°F) and *C* (initial drying in a batch agitated steam drier under a vacuum of 26 in. Hg, 3 h at 130°F; final drying as for *B*). The evaluation followed published methods (Carpenter, Duckworth, Ellinger & Shrimpton, 1952).

Processing method	Meal no.	Type of offal	Gross protein value, chicks (casein = 100)		Selected vitamins: Riboflavin (μg/g dry matter)	Selected vitamins: Vitamin B_{12} (μg/g dry matter)
A. Concentrator and flame-drier	1	Mixed	93	93	(4·7*)	0·077
	2	Cod	92		4·4	0·066
B. Continuous Farramatic	3	Mixed	85	85	5·2	0·081
	4	Cod	85		5·1	0·064
C. Batch-vacuum	5	Mixed	95	93	5·1	0·051
	6	Cod	91		5·9	0·061

* Of the six extracts assayed for each meal two, in this instance, gave unexplained high values not used in the average.

In the chick tests all the meals showed the order of gross protein value expected from the amino-acid composition of this type of material (Carpenter, 1954). More riboflavin appeared to be destroyed in the flame-drying than in the other processes; but protein quality and vitamin B_{12} were at least as high as with the lower temperature methods—as found by Clandinin (1949) and Southcott & Tarr (1953) for herring meals. The slightly darker colour of the flame-dried meals was not, therefore, a reliable indication of lower quality.

The *B* meals had slightly lower gross protein values than the others—an indication possibly of a greater tendency for oxidation in this process. However, small differences may equally be explained by variations in the raw material from batch to batch; and the differences in vitamin content of these meals are not related simply to the processing. Any process may give poor results if improperly operated, but short exposure to high temperatures is not necessarily destructive.

We are grateful for the co-operation of the Hull Fish Meal and Oil Co. Ltd and the Association of Fish Meal Manufacturers.

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Penicillin in Practical Pig Feeding. By W. F. J. CUTHBERTSON and G. A. CHILDS, *Glaxo Laboratories Ltd, Greenford, Middlesex*